DOI:
10.1111/cea.13111

Document Version
Peer reviewed version

CLINICAL & EXPERIMENTAL ALLERGY

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Journal:	*Clinical and Experimental Allergy*
Manuscript ID	CEA-2016-0458.R1
Manuscript Type:	Original Article-Epidemiology of Allergic Disease
Date Submitted by the Author:	n/a
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Keywords:	food allergy, IgE, immunologic tests
Additional Keywords:	peanut exposure, FLG mutation, peanut sensitization

Environmental peanut exposure increases the risk of peanut sensitization in high risk children

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Word count: 2802

Short Title: Environmental peanut exposure and peanut sensitization in BAMSE

Abstract

Background: High household peanut consumption is associated with the development of peanut allergy, especially when peanut allergic cases are compared against atopic controls; thus environmental peanut exposure (EPE) may be a risk factor for peanut sensitization and allergy. In this study we explored the relationship between EPE and peanut sensitization in a population based cohort.

Methods: Maternal bed-dust was collected postnatally and EPE was quantified using a polyclonal peanut ELISA. Peanut sensitization was assessed by peanut specific IgE and peanut protein allergen molecules (PAM) (Ara h 1, 2 or 3 ≥0.35kU/L). Initial nested case control analysis was performed (n=411) followed by whole cohort analysis (n=1878).

Results: There was a significant association between EPE around birth and peanut specific IgE at age 4 years (OR=1.41 95% CI:1.05-1.90, and peanut PAM sensitization at age 8 years (OR=2.11 95% CI:1.38-3.22) compared against controls matched for gender and parental atopy; *FLG* loss-of-function mutations, egg sensitization at age 4 years, infantile eczema and allergic rhinitis were significantly associated with peanut sensitization; however, there was no significant interaction with EPE. When the whole BAMSE cohort was assessed, EPE was no longer associated with peanut sensitization; however, EPE was associated with peanut PAM sensitization when compared against egg-sensitized peanut-tolerant controls adjusted odds ratio of 1.56 per unit EPE (95% CI:1.14-2.12).

Conclusions: EPE was associated with an increased risk of peanut specific IgE and peanut PAM sensitization at 8 years of age when comparing peanut sensitized cases against atopic controls (egg sensitized children or children matched for parental atopy).

Abstract word count: 251

Key words: BAMSE, egg sensitization, environmental peanut exposure, *FLG* mutation, infantile eczema peanut sensitization

Funding

The research was funded/supported by Action Medical Research (S/P/4529) and by the National Institute for Health Research (NIHR) Clinical Research Facility at Guy's & St Thomas' NHS Foundation Trust and NIHR Biomedical Research Centre based at Guy's and St Thomas' NHS Foundation Trust and King's College London. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health. The BAMSE study was funded by the Swedish Research Council, the Stockholm County Council, the Swedish Research Council for Health, Working life, and Welfare the Swedish Asthma and Allergy Foundation, the Swedish Heart and Lung Foundation, and the Gillbergska Foundation.

Abbreviations

AIC: Akaike information criterion

Ara h: Arachis hypogaea

BAMSE: Children/'Barn' Allergy Milieu Stockholm Epidemiological project

CI: confidence intervals

EPE: environmental peanut exposure

FLG: gene encoding the protein filaggrin

GEE: Generalized Estimating Equations

ISAAC: International Study of Asthma and Allergies in Childhood

IQR: Interquartile range

PAM: protein allergen molecule

QIC**:** Quasi Likelihood Independence models criterion

Introduction

Peanut allergy has increased,[1;2] is a leading cause of anaphylaxis in food allergy[3] and has a significant impact on quality of life for the child and their family.[4;5] Most children react on first known oral exposure to peanut; thus sensitization must be occurring earlier.[6;7] Understanding the way children become sensitized to peanut is therefore imperative in order to prevent this condition. Observational[8] and animal work[9] suggests that epicutaneous peanut exposure and early onset severe eczema may play an important role in peanut sensitization.

Epicutaneous exposure may be the route that peanut levels in dust sensitize a child in early life, particularly if the skin barrier is broken through eczema or specific genetic mutations associated with skin barrier dysfunction (e.g. filaggrin (*FLG)* mutations or eczema). We have recently shown in the Manchester Asthma and Allergy Study (MAAS) and Consortium of Food Allergy Research (CoFAR) cohort that *FLG* loss-of-function mutations and eczema severity respectively can increase the impact of early EPE on peanut sensitization and allergy.[10;11].

A dose response relationship has also been demonstrated between household peanut consumption (used as an indirect marker for environmental peanut exposure: EPE) and the risk of developing peanut allergy in young children.[12] They compared household peanut consumption in peanut allergic individuals against children who were at high risk of developing peanut allergy (due to the presence of egg allergy) but had not developed peanut allergy. Household peanut consumption was ten times higher in households with infants with peanut allergy versus children with egg allergy without peanut allergy but was only 3 times higher in non atopic controls, highlighting that household peanut consumption was more likely to be associated with peanut allergy in high risk children.[12] Peanut antigen in the infant's bed-dust and play-area-dust is highly positively correlated with household peanut consumption and stimulates basophils from peanut allergic children, thus has the potential to also sensitize individuals.[13]

Peanut sensitization (specific IgE≥0.35kU/L) is present in up to 10% of children and protein allergen molecule (PAM) analysis of the seed storage proteins in peanut (Ara h 1, 2 and 3) has been shown to improve the specificity and sensitivity of diagnosing peanut allergy.[14;15] In BAMSE, children with IgE reactivity to Ara h 1, 2 or 3 at age 8 years (but not Ara h 8) reported peanut allergic symptoms in 87% of cases, whereas children with IgE reactivity exclusively to Ara h 8 reported peanut allergic symptoms in only 17% of cases which were also milder.[16] Sweden has high levels of birch pollen sensitization which explains the Bet v 1 in-vitro cross-reactivity with peanut Ara h 8;[16;17] thus, in this study we differentiated between primary peanut sensitization (IgE reactivity to Ara h 1, 2 or 3) and pollen cross-reactivity (IgE reactivity to Ara h 8).

This study aimed to assess whether EPE (as defined by peanut protein levels in household dust) is a risk factor for the development of peanut sensitization. The analysis was done in two steps; firstly among high risk children, in a nested cases analysis and secondly among the whole studied population (a cohort analysis). Given previous findings we also wanted to ascertain the modifying effects of *FLG* loss-of-function mutations, infantile eczema and preceding egg sensitization.

Methods

The BAMSE study is an unselected Swedish population birth cohort. The design and methodology are described elsewhere.[18] In brief 4089 unselected newborn children were recruited from 1994-1996 and have been evaluated for various health conditions over time. No intervention was performed. The BAMSE study obtained ethical approval for environmental sampling of dust.

Demographics

Serum specific IgE to peanut (ImmunoCAP system, ThermoFisher, Sweden) was measured at 4 and 8 years and children were defined as 'peanut sIgE sensitized' if peanut specific IgE was ≥0.35kU/L. Peanut allergen molecules (PAM) were assessed at 8 years (ImmunoCAP system, ThermoFisher, Sweden) and children were considered to be peanut PAM sensitized if *Ara h 1, 2 or 3 was ≥0.35kU/L and not peanut PAM sensitized if Ara h 1, 2 and 3 were <0.35kU/L or peanut sIgE was <0.35kU/L at 8 years (where no peanut PAM available).* Children with positive peanut specific IgE at 8 years of age but with no available PAM analysis were excluded from this analysis. Egg sensitization was defined as egg specific IgE ≥0.35kU/L at 4 years of age.

Parental atopy was defined as a doctor's diagnosis of asthma and prescription of asthma medication and/or a doctor's diagnosis of hay fever in combination with furred pets- and/or pollen allergy at the time of questionnaire.

Genotyping was performed for *FLG* mutations common in Scandinavia by using TaqMan allelic discrimination assays for R501X and R2447X and matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry for 2282del4. Children with a mutation in any of these positions were classified as having a loss-of function mutation.

A reported history of infantile eczema was assessed by questionnaire and was defined as dry skin, itchy rashes for 2 weeks or more and age specific localization (face or arms/legs extension surfaces or arms/legs flexures or wrists/ankles flexures) and/or doctor's diagnosis of eczema during the first year of life.

Ethnicity was based on where the parents were born. Allergic rhinitis at 4 and 8 years was assessed using International Study of Asthma and Allergies in Childhood (ISAAC) validated questionnaires, and was defined as persistent rhinitis without a common cold the last 12 months before 4 and 8 years respectively.[19]

Dust collection methodology

Dust samples were collected at a median of 2 months of age from the mother's mattress. The mother's mattress was vacuumed for 2.5 minutes with a small disposable filter bag (Allergy Control Products Inc. Ridgefield CT, USA) inserted in the front hose of the vacuum. The dust containing filter bags were sealed in plastic bags and stored at -20ºC. Dust samples were sieved and fine dust was weighed and extracted in proportional volumes of extraction solution as previously described.[20] Peanut protein in dust was determined using the Veratox polyclonal ELISA against whole peanut protein (Neogen Corporation, Lansing, MI, USA). The lower limit of quantitation (LLQ) of the assay was defined as 100ng/ml and samples below this value were defined as 50ng/ml (LLQ/2).[21] Results were converted from ng/ml into µg peanut protein/gram dust. Due to batch to batch variability of the Veratox ELISA results were batch corrected prior to being entered into the final statistical analysis.

Statistical analysis

Data was entered into an SPSS (SPSS 19.0; SPSS Inc, Chicago, IL, USA) and STATA spreadsheet (Timberlake Consultants Ltd London, UK) for the purpose of analysis. Peanut protein levels in dust (µg/gram) underwent natural log transformation. Initial nested case control analysis was performed with peanut sIgE sensitized cases at 4 years of age matched for gender and parental atopy with a 2:1 control (n=274) to case (n=137) matching. Children with peanut PAM sensitization defined by Ara h 1, 2 or 3 ≥0.35kU/L at 8 years of age were also matched against controls at age 8 years matched for gender and parental atopy with a 2:1 ratio of controls (n=130) to cases (n=65). Conditional logistic regression (incorporating matching) was performed for the case control analysis

using robust standard error for peanut sIgE sensitization at 4 years and peanut PAM sensitization at age 8 years.

Subsequently analysis was performed for the relationship between early EPE and peanut sensitization in all children from the BAMSE cohort with available postnatal maternal bed-dust and *FLG* genotyping (n=1878). Logistic regression analysis was performed for peanut PAM sensitization at 8 years of age. Factors associated with peanut sIgE sensitization were assessed using Generalized Estimating Equations (GEE) with an exchangeable working correlation matrix to account for repeated measures within individuals at 4 and 8 years. Univariate followed by multivariate regression analysis was performed including EPE, *FLG* loss-of-function mutations, infantile eczema and egg sensitization at 4 years of age plus other covariates significantly association with peanut sensitization ($P \leq .05$) which also improved the quality of fit of the multivariate model using the Akaike information criterion (AIC) for logistic regression and the Quasi Likelihood Independence models criterion (QIC) for GEE analyses.

Where the *FLG* mutation covariate was entered into the statistical model, participants with missing ethnicity data (35/1878=1.9%) and non-caucasians (165/1842=8.8%) were excluded as distinct *FLG* loss-of-function mutations are present in different populations and the *FLG* loss-of-function mutations assessed in this study have only been associated with eczema in Caucasian European populations [22;23] Peanut levels in dust (µg/g) were compared between groups using the Mann-Whitney U test. Proportions between groups (e.g. infantile eczema and *FLG* mutations) were compared using Pearson chi-squared. Statistical significance was assessed at $P < .05$.

Results

Details of demographics and clinical characteristics included on this study are described in Table EI. Median peanut protein [IQR] in dust was 4.07µg/gram [1.58, 11.86]. Peanut extract sIgE sensitization was 6.6% at 4 years (n=103/1572) and 8.6% at 8 years (n=161/1876). Peanut PAM sensitization at 8 years was 4.1% (n=75/1854), of which 74/75 (99%) had Ara h 2 ≥0.35kU/l. We first performed a nested case control analysis, and based on positive findings for this subsequently proceeded to then analyse the whole BAMSE cohort.

Nested case control analysis for peanut sIgE and PAM sensitization

We compared peanut sensitized children against children without peanut sensitization matched for gender and parental atopy as described in the statistical methods; EPE was a risk factor for both peanut extract sIgE sensitization at 4 years and peanut PAM sensitization at 8 years with a 23% and 29% increased risk of peanut sensitization per natural log (*ln*) unit increase in EPE respectively (Table I). In children who were peanut extract sIgE sensitized but not peanut PAM sensitized, there was only borderline significance towards an association between EPE and peanut sensitization (OR=1.20, 95% CI: 0.97-1.48).

Differential relationship between EPE and primary peanut PAM sensitization and non-clinically significant peanut sensitization is displayed in Figure 1. Median peanut protein in dust was higher in peanut extract sIgE sensitized children (3.39 µg/g, IQR 1.41-11.01, n=137) than non-peanut extract sIgE sensitized controls (2.28µg/g, IQR 0.88-5.14, n=274) (*P*<.01); and higher in peanut PAM sensitized children (4.64µg/g, IQR 1.58-12.77, n=65) than non-peanut PAM sensitized controls (2.24µg/g, IQR 0.88-4.74, n=130) (*P*<.01).

On multivariate conditional logistic regression analysis, EPE was significantly associated with peanut specific IgE and peanut PAM sensitization with a 1.41- and 2.1-fold increased risk per unit increase EPE respectively (Table II).

Infantile eczema and egg sensitization were both significantly associated with peanut extract sIgE and PAM sensitization (Table II). *FLG* loss-of function mutations increased the risk of peanut

specific IgE 3.78-fold and peanut PAM sensitization 7.33-fold. Allergic rhinitis at age 4 and 8 years was associated with both peanut specific IgE at age 4 years and peanut PAM sensitization at age 8 years in the multivariate analysis. The number of biological siblings, duration of exclusive and total breastfeeding and maternal age at baseline were not risk factors for peanut sensitization. There was no significant interaction between EPE and either infantile eczema, egg sensitization or *FLG* loss-of-function mutations on peanut PAM sensitization (data not shown).

Whole cohort analysis for peanut sIgE and PAM sensitization

In a second step following positive findings obtained on case control analysis, analysis was performed for the relationship between early EPE and peanut sensitization in all children from the BAMSE cohort with available postnatal maternal bed-dust and *FLG* genotyping (n=1878). On both univariate (Table III) and multivariate analysis (Table IV) infantile eczema, egg sensitization, *FLG* loss-of function mutation and allergic rhinitis at age 8 years were associated with peanut extract sIgE at age 4 and 8 years. For PAM sensitization at age 8 significant associations were also seen on univariate (Table III) and multivariate analysis (Table IV) for infantile eczema, egg sensitization and allergic rhinitis at age 8 years. However, EPE was no longer associated with peanut sensitization. There was no interaction between EPE and *FLG* mutations, infantile eczema, egg sensitization or parental atopy on peanut sensitization.

Subgroup analysis of peanut PAM sensitization against egg sensitized non peanut allergic individuals

On subgroup analysis we compared peanut PAM sensitized children (n=75) against children who were sensitized to egg (sIgE≥0.35kU/L at age 4 years) but did not go on to develop peanut PAM sensitization (n=59). EPE was a risk factor for peanut PAM sensitization on both univariate (OR=1.33 95% CI:1.01-1.74, *P*<.05, n=134) (Table V) and multivariate analysis (OR=1.56, 95%

CI:1.14-2.12, P<.01, n=132, AIC: 331) adjusting for allergic rhinitis at age 8 years and infantile eczema. Peanut levels in household dust were also significantly higher in peanut PAM sensitized children (median 4.79µg/g, IQR 1.63-12.00) versus egg sensitized, non-peanut PAM sensitized children (median 2.35µg/g, IQR 1.23-6.15) (*P*=.03).

We assessed the impact of EPE on peanut sensitization when using other high-risk groups of children who did not become peanut sensitized (*FLG* mutations, infantile eczema, parental atopy) and there was no significant association.

Discussion

In the prospective birth cohort BAMSE, there was an exposure-response relationship between EPE (quantified using peanut protein levels from maternal mattress-dust postnatally) and peanut PAM sensitization (Ara h 1, 2 or 3 ≥0.35kU/L) among high risk children (in a nested cases analysis). When all children in the whole studied population were assessed, there was no association between EPE and peanut sensitization; however, on subgroup analysis there was an exposure-response relationship between EPE and peanut PAM sensitization when compared against high risk egg sensitized children.

This supports the findings by Fox et al. where household peanut consumption during the first year of life (as an indirect marker of EPE) was higher in peanut allergic cases than in egg allergic controls.[12] The exposure-response relationship between EPE and PS (when compared against atopic controls) was stronger between EPE and peanut CRD sensitization (Ara h 1,2 or 3≥0.35kU/L) than between EPE and non-clinically relevant PS (peanut sIgE ≥0.35kU/L but negative results for Ara h 1,2 or 3<0.35kU/L) (Figure 1). The association between household peanut consumption and peanut sensitization was also found in a study by Garcia-Boyano et al. [24] and supports the concept that EPE increases the risk of clinically relevant peanut sensitization.

In other cohorts we have shown a synergistic effect between EPE and markers of an impaired skin barrier (infantile eczema and *FLG* loss-of-function mutations) on peanut sensitization and allergy.[11;25] Although in BAMSE we did not find significant interactions between EPE and *FLG* mutations or infantile eczema on peanut sensitization, we did show on subgroup analysis that early EPE has more impact in children with certain risk factors for the development of peanut sensitization (such as parental atopy or egg sensitization) than children without these risk factors. The BAMSE cohort is relatively non-atopic, being from the general population in Sweden, which may have contributed to lower strength of associations between EPE and atopic markers.

Infantile eczema was one of the most important risk factors for peanut sensitization in BAMSE; this association has been cited in numerous studies.[8;26] *FLG* loss-of-function mutations were less important than infantile eczema in predicting the development of peanut sensitization, and *FLG* mutations became more important if infantile eczema was not included in the regression model. Previous studies have found that children that carry a *FLG* mutation have an increased risk of peanut allergy even after adjusting for eczema.[27] In this study eczema seemed to be the overriding factor for peanut sensitization; however, it should be noted that *FLG* mutations were quite low in BAMSE (7.14%) in comparison with other population-based cohorts.[22;27] Egg sensitization was highly associated with peanut sensitization; which has previously been shown in numerous cohort studies;[25;28;29] it may be that egg sensitization shows a predisposition to mount allergic antibodies to other allergens as egg allergy is highly associated with the development of other food allergies, asthma and aeroallergen sensitization.[30;31] Observational studies have shown that in children with severe, early onset eczema, up to 50% are sensitized to egg;[26] thus egg sensitization may be a marker of eczema severity.

Limitations of this study included the lack of diagnostic food challenges to determine peanut allergy; however, peanut PAM sensitization has been shown to be a much more accurate predictor of clinically confirmed peanut allergy than peanut specific IgE.[32;33] Caucasian ethnicity was defined as being born in Sweden as between 1994-1996 (when the BAMSE cohort commenced), most participants born in Sweden would have been of Northern European descent. In addition participants from Finland, Greece or Eastern Europe who were not born in Sweden were also defined as Caucasian.[34] Findings on case control analysis could not be replicated on whole cohort analysis, therefore these findings need to be confirmed in other population based cohorts. Strength of the present study includes the population based design and relatively high number of study participants.

Conclusion

In summary, although EPE did not increase the risk of peanut sensitization in children who did not already have atopic risk factors, the findings from the BAMSE cohort support the hypothesis that in specific high risk groups, peanut levels in dust around the time of birth may pose a risk for later

peanut sensitization. Further prospective studies are needed to confirm these findings in other populations.

Acknowledgement

We wish to thanks Dr Alick Stephens for his assistance in the BAMSE dust analysis. The authors thank all children and their parents in the BAMSE cohort

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Ref Type: Abstract

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Table EI: Demographics of whole studied BAMSE population with available dust and *FLG* genotyping (n=1878)

	Number of cases	No. data points	Percentage
Peanut specific IgE ≥ 0.35kU/L at 4 years	103	1572	6.55%
Peanut specific IgE ≥ 0.35kU/L at 8 years	161	1876	8.58%
Ara h 1, 2 or 3 ≥0.35kU/L at 8 years vs. Ara h 1, 2 or 3 <0.35kU/L or peanut sIgE <0.35kU/L (excluding peanut sIgE ≥0.35kU/L with no components)	75	1854	4.05%
Avoiding peanut because of previous adverse reaction	73	1878	3.89%
History of infantile atopic dermatitis	313	1840	17.01%
At least one *FLG* mutation (R501X, 2282del or R2447x)	134	1878	7.14%
Allergic rhinitis (ISAAC definition) (age 4 years)	215	1828	11.76%
Allergic rhinitis (ISAAC definition) (age 8 years)	288	1865	15.44%
Egg sIgE≥ 0.35IU/ml at 4 years of age	82	1575	5.21%
Male gender	973	1871	52.00%
Caucasian ethnicity	1677	1842	91.04%
Biological siblings	1695	1870	90.64%
Parental atopy – (asthma/AR/AD)	838	1858	45.10%
Maternal age (mean, SD)	30.9 (SD4.49)	1871	N/A
Peanut protein in maternal bed-dust (µg/g): median [IQR]	4.07 [1.58-11.86]	1878	N/A
Exclusive breast-feeding duration (months): mean (SD)	5.19 (2.43)	1838	N/A
Total breast-feeding duration (months): mean (SD)	8.73 (3.31)	1825	N/A

Table I: Univariate conditional logistic regression incorporating matching for peanut sensitized cases versus non-sensitized controls matched for gender and parental atopy. Peanut sIgE sensitization (≥0.35kU/L) at age 4 years and peanut PAM sensitization (Ara h 1, 2 or 3 ≥0.35kU/L) at age 8 years.

	Peanut sIgE sensitized at age 4 years (n=411)			Peanut PAM sensitized at age 8 years (n=195)		
	OR	95% CI	*P* value	OR	95% CI	*P*-value
EPE (µg/gram ln transformed)	1.23	1.06-1.43	**<.01**	1.29	1.04-1.61	**.02**
No. of biological siblings	1.38	0.63-3.03	.42	2.50	0.77-8.16	.13
≥1 *FLG* loss-of-function mutation (excluding non-caucasians)	2.67	1.18-6.04	**.02**	3.00	0.84-10.75	.09
History of infantile eczema	7.35	4.20-12.83	**<.001**	6.39	2.95-13.83	**<.001**
Egg sIgE sensitization: 4 years	18.91	7.50-47.66	**<.001**	12.85	3.78-43.67	**<.001**
Allergic rhinitis: 4 years (ISAAC)	3.44	1.90-6.23	**<.001**	2.53	1.04-6.18	**.04**
Allergic rhinitis: 8 years (ISAAC)	11.44	5.68-23.07	**<.001**	12.97	4.54-37.05	**<.001**
Exclusively breastfed (months)	0.95	0.88-1.03	.22	0.98	0.87-1.11	.77
Breastfed (excl/partial) (months)	0.99	0.93-1.05	.74	1.01	0.93-1.11	.76
Maternal age at baseline (in years)	0.99	0.94-1.04	.65	1.05	0.99-1.12	.12

Table II: Multivariate conditional logistic regression incorporating matching for peanut sensitized cases versus non-sensitized controls matched for gender and parental atopy. Peanut sIgE sensitization (≥0.35kU/L) at age 4 years and peanut PAM sensitization (Ara h 1, 2 or 3 ≥0.35kU/L) at age 8 years. Non-caucasians excluded due to inclusion of *FLG* mutations as covariate.

	Peanut sIgE sensitized at age 4 years (excluding non-caucasians) (n=237) *AIC: 90.78			Peanut PAM sensitized at age 8 years (excluding non-caucasians) (n=143) *AIC: 49.19		
	OR	95% CI	*P* Value	OR	95% CI	*P* Value
EPE (µg/gram ln transformed)	1.41	1.05-1.90	**.02**	2.11	1.38-3.22	**.001**
≥1 *FLG* loss-of-function mutation	3.78	0.93-15.36	.06	7.33	1.21-44.21	**.03**
History of infantile eczema	2.53	0.98-6.51	**.05**	3.93	1.03-15.00	**.05**
Egg sIgE sensitization: 4 years	28.00	5.24-149.39	**<.001**	39.9	1.47-1081.68	**.03**
Allergic rhinitis: 8 years (ISAAC)	12.57	4.164-37.98	**<.001**	28.93	3.77-221.61	**<.001**

*Smaller AIC is better

Table III: Univariate analysis of peanut specific IgE sensitization aged 4 and 8 years using GEE to account for repeated measures within individuals at 4 and 8 years and logistic regression for peanut PAM sensitization at age 8 years in the whole BAMSE cohort (n=1878).

	Univariate GEE for peanut sIgE sensitization at 4 and 8 years			Univariate peanut PAM sensitization at age 8 years		
	OR	95% CI	*P* Value	OR	95% CI	*P* Value
EPE (µg/gram ln transformed)	0.95	0.86-1.06	.39	1.01	0.86-1.19	.89
Gender	1.14	0.83-1.56	.43	1.41	0.88-2.26	.15
Full older siblings	1.08	0.63-1.85	.77	0.86	0.40-1.81	.68
≥1 *FLG* loss-of-function mutations (excluding non-caucasians)	1.85	1.08-3.16	**.03**	1.76	0.82-3.77	.15
History of infantile eczema	6.49	4.66-9.02	**.001**	7.94	4.91-12.83	**<.001**
Egg sIgE sensitization at 4 years	13.58	8.55-21.58	**<.001**	11.04	6.13-19.90	**<.001**
Allergic rhinitis aged 4 years (ISAAC)	2.82	1.92-4.13	**<.001**	2.16	1.22-3.83	**<.01**
Allergic rhinitis aged 8 years (ISAAC)	8.05	5.78-11.22	**<.001**	5.87	3.66-9.42	**<.001**
Parental atopy (asthma and/or hay-fever)	1.77	1.28-2.44	**<.001**	1.77	1.10-2.83	**.02**
Non-caucasian ethnicity	1.58	0.97-2.57	.07	1.09	0.49-2.43	.79
Exclusively breastfed in months	0.98	0.94-1.06	.92	0.97	0.88-1.06	.49
Breastfed (exclusive/partial) in months	0.99	0.94-1.03	.57	0.97	0.91-1.04	.43
Maternal age at baseline (in years)	1.02	0.98-1.05	.44	1.02	0.97-1.07	.53

Table IV: Multivariate analysis of peanut specific IgE sensitization aged 4 and 8 years using generalised estimating equations (GEE) to account for repeated measures within individuals at 4 and 8 years and logistic regression analysis for peanut PAM sensitization at age 8 years in the BAMSE cohort. excluding non-caucasians. Goodness of fit assessed by QIC and AIC.

	Multivariate peanut sIgE sensitization (excluding non-caucasians) (1388) QIC:1094			Multivariate peanut PAM sensitization (excluding non-caucasians) (n=1375) AIC:767		
	OR	95% CI	*P* Value	OR	95% CI	*P* Value
EPE (µg/gram ln transformed)	0.98	0.84-1.13	.75	1.08	0.88-1.32	.48
≥1 *FLG* loss-of-function mutations	2.37	1.22-4.60	**.01**	1.82	0.72-4.57	.20
History of infantile eczema	3.81	2.44-5.94	**<.001**	5.51	3.03-10.03	**<.001**
Egg sIgE sensitization at 4 years	8.12	4.35-15.15	**<.001**	6.61	3.16-13.80	**<.001**
Allergic rhinitis aged 8 years (ISAAC)	5.57	3.57-8.71	**<.001**	3.05	1.65-5.63	**<.001**

Table V: Univariate analysis of factors associated with peanut PAM sensitization (at age 8 years) versus non peanut PAM sensitization (at age 8 years) in high risk children with preceding egg sensitization (at age 4 years) (n=134).

	Peanut PAM sensitization vs. non-peanut sensitized egg sensitized controls		
	OR	95% CI	*P* Value
EPE (µg/gram ln transformed)	1.33	1.01-1.74	**<0.05**
Gender	1.90	0.95-3.80	0.07
Full older siblings	0.61	0.17-2.13	0.44
≥1 *FLG* loss-of-function mutations (excluding non caucasians)	1.09	0.33-3.56	0.89
History of infantile eczema	2.51	1.24-5.08	**0.01**
Allergic rhinitis aged 4 years (ISAAC)	0.87	0.38-1.96	0.73
Allergic rhinitis aged 8 years (ISAAC)	2.65	1.26-5.56	**0.01**
Parental atopy (asthma and /or hay-fever)	1.34	0.67-2.65	0.41
Non-caucasian ethnicity	2.55	0.93-6.98	0.07
Exclusively breastfed in months	0.89	0.77-1.04	0.14
Breastfed (exclusive/partial) in months	0.99	0.89-1.09	0.76
Maternal age at baseline (in years)	1.06	0.99-1.14	0.11

Legend

Figure 1: Mean predictive probability of peanut sensitization in children with primary peanut PAM sensitization (Ara h 1,2 or 3 ≥0.35kU/L) versus children with peanut specific IgE ≥0.35kU/L but with negative results for peanut PAM (Ara h 1,2 or 3<0.35kU/L).

Contributions of each author

HB, IK, EH, MW, GL had substantial contributions to the conception and design of the study. HB, KM, MP and VR made substantial contributions to acquisition of peanut-dust data and analysis. HB and AD performed the statistical analysis of results. IK, EH, CS, AB, EM and MW had substantial contribution to the BAMSE study and obtaining data to perform this study. CS performed the FLG genotyping. All authors contributed to the drafting and revising the manuscript for intellectual content and have approved the version to be published.

Conflict of interests

H. A. Brough has received research support from the National Peanut Board and has received research support, lecture fees, and travel support from Thermo Fisher Scientific. G. Lack and V. Turcanu have received research support from the National Peanut Board. I Kull, K Makinson, E Hallner, C Söderhäll, A Douiri, M Penagos, E Melén, A Bergström and M Wickman declare that they have no relevant conflicts of interest.

Comments for Authors:
This interesting article in which the authors to investigated whether epicutaneous peanut exposure is related to peanut (component) sensitization at year 4 and year 8. This work is important and relevant to explore a possible relationship between the household peanut consumption and the development of peanut allergy especially as it is investigated in an unselected birth cohort. However, I have concerns about the methodology used and therefor about the interpretation and conclusions drawn from the results of this article.

Major points:

- Was the stepwise data analysis (a nested case control analysis followed by whole cohort analysis) chosen for (cost)-efficiency or other reasons? Please explain, because if possible and data are available you should prefer to use the whole cohort analysis to answer your research questions properly.

The reviewer is correct that the stepwise data analysis (a nested case control analysis followed by whole cohort analysis) was chosen for financial and logistical reasons. Both the peanut levels in dust and filaggrin analysis had not yet been performed thus required the finances and manpower to run these analyses. We wanted to see if there was a difference in peanut levels in dust between peanut sensitised cases and high risk controls (matched for parental atopy) (n=411) before proceeding with the whole cohort (n=1878). We have stated 'The analysis was done in two steps for financial and logistical reasons' into the last paragraph of the introduction (line (track changes manuscript) 130) to highlight this.

- I think the OR's throughout the article should be interpreted with care here as an association was found and no causation. It would be better to speak about an increased probability of developing sensitization to peanut with each unit increase of EPE instead of a x% risk for peanut sensitization.

We agree with the reviewer that the OR represent association and not causation. We have therefore amended the text as suggested throughout the manuscript.

- Several confounders should be addressed that can influence the association found between EPE and peanut sensitization: the severity of the child's eczema, the use of emollients and / or topical steroids, parental atopy (including AD), parental

peanut / food allergy, other food allergies in the child (besides egg allergy), oral exposure to peanut).

Thank-you for suggesting these potential confounders. We will discuss each in turn including their association with peanut sensitization at age 8 years:

1) *In BAMSE the severity of the child's eczema was assessed by the extent of localisation reported by the parents in the questionnaire at 1 year of age. The extent of localisation of eczema was up to 10 areas (face, scalp, arms/legs, armpits, flexures, wrists/ankles, neck, chest/belly or back, front body inside nappy, buttocks). The extent of localisation of infantile eczema was associated with peanut sensitization on univariate analysis (Table III and V) but was no longer significantly associated on multivariate analysis. There was no significant interaction between EPE and extent of eczema localisation on peanut sensitization.*

2) *Use of topical steroids was reported by parents within the last year (from age 1-2 years) at the 2 year questionnaire. Use of topical steroids was associated with peanut sensitization on univariate analysis (Table III and V) but was no longer significantly associated on multivariate analysis. There was no significant interaction between EPE and use of topical steroids on peanut sensitization.*

3) *We included both definitions of parental atopy (with and without eczema) in the demographics Table E1, methods section and results section for the whole cohort analysis. On univariate logistic regression analysis (Table III), the association between parental atopy (excluding eczema) and primary peanut sensitization at age 8 years was significant (OR 1.77 (95% CI: 1.1-2.83) p=0.02), whereas the association between parental atopy (including eczema) was not significant (OR 1.50 (95% CI: 0.95-2.39), p=0.09). We postulate that adult eczema may be non-atopic and therefore is not related to an increased risk of atopy in the child. To demonstrate thus we evaluated the rate of eczema within the first year of life in children with parental atopy that did or did not include eczema. We found that 24% (143/595) of children with parental atopy excluding eczema had infantile eczema whereas only 22.5% (185/823) of children with parental atopy including eczema had infantile eczema. We therefore used the definition of parental atopy excluding eczema*

throughout the rest of the manuscript. We have explained this in the methods section (line (track changes manuscript) 160-165):

'This definition of parental atopy was selected rather than the same definition with inclusion of a doctor's diagnosis of eczema (excluding contact dermatitis); this was because the children of parents with atopy including eczema were less atopic (lower infantile eczema) and parental atopy including eczema was not associated with the outcome of interest (primary peanut sensitization at 8 years of age); this is probably because adult eczema is often non-atopic.'

4) *The BAMSE study does not have information on parental peanut or food allergy or maternal peanut consumption during pregnancy or breastfeeding.*

5) *The BAMSE study does not have information on oral consumption of peanut in the child in the first year of life.*

6) *The association between other food allergies in the child (besides egg allergy) and primary peanut sensitization at age 8 years was assessed using parental questionnaires at the 8 year visit where the parents were asked: 'Is your child allergic to any foodstuff?' An adverse reaction included itching in the mouth, nose/eye problems, trouble breathing, vomiting or diarrhoea, eczema or nettle rash. Parents were then asked the same questions about specific foods: milk, egg, fish, shell-fish, wheat, soy, apple, peach, kiwi, avocado, raw carrot, banana, nuts/almond (except peanut). Results are shown in Table III, IV and V in the manuscript. Although the majority of the adverse reactions to foods at age 8 were associated with peanut sensitization, on multivariate analysis only tree nut and soya adverse reactons were significantly associated.*

- To answer the question whether FLG loss-of-function mutations, infantile eczema and preceding egg sensitization have modifying effects on the association between EPE and peanut sensitization selective samples based on the outcome were drawn from the cohort (peanut PAM sensitized versus egg sensitized children). However, to conclude whether those proposed factors are effect modifiers, the association between EPE and the outcome should be investigated in both strata (with and without the possible effect modifier). For example it should be investigated if there is a difference in the association between EPE and peanut sensitization in children with eczema compared to children without eczema.

The reason we analysed the level of EPE in peanut sensitized versus egg-sensitized, peanut-tolerant individuals was because we wanted to mimic the analysis by Fox et al (J Allergy Clin Immunol 2009;123:417-23) who compared level of household peanut consumption in peanut allergic cases versus egg allergic non-peanut allergic infants. In the study by Fox et al. it was shown that household consumption of peanut (as a marker of environmental exposure to peanut) was associated with peanut allergy especially when compared against egg allergic, non-peanut allergic controls.

We assessed whether there was an interaction between EPE and peanut sensitization in children with or without infantile eczema, egg sensitization, FLG mutations and parental atopy and there were no significant associations.

Abstract

- Line (track changes manuscript) 35 Please explain what kind of statistical analysis are performed (dependent / independent variables and which kind of modelling) in which subgroup.

We have provided an overview of the dependent (outcome) variable and main independent variables (EPE) for the case control, cohort and subgroup analysis and have stated which statistical analysis was used (line (track changes manuscript) 36-43):

'Initial nested case control analysis was performed comparing peanut sensitized cases against high-risk controls (matched for parental atopy) (n=411) using a conditional regression analysis. This was followed by whole cohort analysis (n=1878) comparing EPE against peanut sIgE sensitization at ages 4 and 8 years using Generalized Estimating Equations and against primary peanut sensitization at age 8 years using a logistic regression model. Finally, a subgroup analysis was performed comparing the impact of EPE in peanut sensitized versus egg-sensitized peanut tolerant individuals using logistic regression analysis. Levels of EPE were compared between groups using the Mann-Whitney U test.'

- Line (track changes manuscript) 37 I find this sentence difficult to interpret. Do you mean that a higher level of EPE (in µg/gram? Or the highest quartile compared to the lower quartiles?) around birth was significantly associated with the presence of peanut sensitization (>0.35kU/L) at year 4?

We mean that a higher level of EPE around birth (in µg/gram) is associated with the presence of peanut sensitization at age 4 years (we did not compare quartiles). We have amended the sentence to make this clearer as follows (line (track changes manuscript) 45-48):

'In the nested case control analysis, a higher level of EPE around birth was associated with the presence of peanut specific IgE sensitization at age 4 years (OR=1.41, 95% CI:1.05-1.90), and primary peanut sensitization at age 8 years (OR=2.11, 95% CI:1.38-3.22) compared against high-risk controls.

Introduction

- Line (track changes manuscript) 110 What do you mean with a high risk population, did you select children with certain risk factors for peanut sensitization for this analysis?

We determined these children to be at higher risk of developing peanut allergy as the controls were matched to the higher level of parental atopy that was present in the primary peanut sensitised cases than in the population overall (46.7% vs. 32.6%). This clarification has been added to the methods (line (track changes manuscript) 207-210).

'Given that these controls were matched for the higher levels of parental atopy in the primary peanut sensitized cases these controls were deemed to be high-risk as the primary peanut sensitized cases had higher levels of parental atopy than the population overall (46.7% vs. 32.6%).'

Methods

- Did you consider to take a lower limit of sIgE sensitization (especially to peanut components) as clinically relevant, e.g. > 0.1 kU?

The results for peanut specific IgE were reported with a cut-off of 0.35kU/l; however, Ara h 1,2 and 3 were reported with a lower cut-off of 0.1kU/l. There were 11 data points that became positive when we redid the analysis using a cut-off of 0.1kU/L instead of 0.35kU/l. We redid the analysis using primary peanut sensitization (Ara h 1,2 and/or 3) with a cut-off of 0.1kU/l and there was no association between EPE and primary peanut sensitization at age 8 years and the other covariate associations did

not significantly change therefore we have kept the analysis using a cut-off for Ara h 1,2 and/or 3 of 0.35kU/l.

- Line (track changes manuscript) 126 Regarding the non-available PAM analysis data: in how many cases occurred missing data?

In line (track changes manuscript) 126 we stated 'children with positive peanut specific IgE at 8 years of age but with no available peanut protein component allergen analysis were excluded from this analysis.' There were 24 children with missing data for this analysis because no serum was available to perform this. This has now been included in the manuscript of the text (line (track changes manuscript) 151-153).

'Twenty-four (1.3%) children with positive peanut specific IgE at 8 years of age but with no available serum for peanut protein component allergen analysis were excluded from this analysis.'

- Was there a selection of children in which PAM analysis was performed and can this have influenced your results?

All children sensitized to peanut at age 8 years, irrespective of sensitization to birch had analysis of Ara h 1,2,3,8 and 9 where enough sample was available. In addition, those children who were peanut sensitized at age 4 years but lost their sensitization at age 8 were also analysed for these component allergens. All children with negative peanut sIgE at age 8 years were assumed to have negative component allergens to peanut. We believe this selection is broad enough so as not to have biased our results. This information has been added to the manuscript (line (track changes manuscript) 142-148).

'Peanut protein component allergens Ara h 1,2,3,8 and 9 were assessed at 8 years (ImmunoCAP system, ThermoFisher, Sweden) in all children sensitized to peanut at age 8 years, irrespective of sensitization to birch. In addition, those children who were peanut sensitized at age 4 years but lost their sensitization at age 8 were also analyzed for these allergens. All other children with negative peanut sIgE at age 8 years were assumed to have negative component allergens to peanut.'

- Line (track changes manuscript) 128 Why was eczema not taken in to account in the parental atopy definition?

We have included both definitions of parental atopy (with and without a history of eczema) in the demographics Table E1, methods section and results section for the whole cohort analysis. Please see response to previous question by the same reviewer.

- How was the presence of a furred pets- and / or pollen allergy determined?

'Doctor diagnosed furred pets- and / or pollen allergy' was self-reported by the mother and/or father for the parental atopy definition in the BAMSE questionnaire.

- Was the GEE modelling only used to select possible predictors of the presence of peanut sensitization? And why did you choose for GEE with repeated measures and not for one logistic regression model with any sensitization to peanut at age 4 and/or age 8 as outcome measure?

GEE modelling was used to select possible predictors of the presence of peanut sIgE sensitization at age 4 and 8 years using first a univariate then multivariate analysis. In the multivariate analysis variables were only included if were significantly associated with peanut sensitization ($p \leq 0.05$) and if their inclusion also improved the quality of fit of the multivariate model using Quasi Likelihood Independence model criterion (QIC) or Akaike information criterion (AIC).

We used GEE with repeated measures rather than one logistic regression model with any sensitization at peanut at age 4 or age 8 as the outcome measure to account for missing data. GEE has built in imputation for missing results using the maximum likelihood analysis. This accounts for bias that could be introduced as if the child is peanut sensitized at age 4 and data is missing at age 8 they would be presumed to be peanut sensitized but if they were not peanut sensitized at age 4 and data was missing at age 8 years they would be excluded from the analysis.

Results

- Table I it would be interesting to split this table according to outcome

Table 1 is split according to the outcome at 4 and 8 years. Please advise if you would like us to change the structure of the Table.

- Table II why was allergic rhinitis at year 8 entered in the model instead of year 4 (as the outcome was assessed at year 4)?

Allergic rhinitis at age 8 years was selected as it improved the fit of the conditional logistic regression model (AIC) better than allergic rhinitis at age 4 years. As described in the statistical methods 'In the multivariate analysis variables were only included if were significantly associated with peanut sensitization (p≤0.05) on univariate analysis and if their inclusion also improved the quality of fit of the multivariate model using Quasi Likelihood Independence model criterion (QIC) or Akaike information criterion (AIC). This clarification has been added as a footnote to Table II.

- Table E1 Almost 4 % of patients is already avoiding peanut due to a previous adverse reaction. At which point in time was this question asked?

This question was asked at age 8 years; however, we realised that a slightly different question was more likey to indicate relevant peanut allergy. Parents were asked 'Is your child allergic to any foodstuff?' which encompassed nose and eye symptoms, itch, breathing problems, diahhroea, eczema, rash, ' then were asked about specific foods: 'Any reaction to peanut?'. The question about peanut avoidance did not encompass any allergic symptoms.

Also 'Any reaction to peanut' and was much more highly associated with primary peanut sensitization (OR 157.6 95% CI: 81-305), p<0.0001) than 'avoiding peanut because of adverse reaction' (OR 51.2 95% CI: 29-91, p<0.0001), therefore we used the definition 'Any reaction to peanut' and also used this definition for other food allergies.

Do you have more information on the presence of clinical relevant peanut allergy?

Unfortunately no diagnostic oral food challenges were performed as part of the BAMSE study as the study was not designed to assess peanut allergy.

- Table E1: parental atopy AD seems included in contrast to the definition described in the method section of the article.

We thank the reviewer for pointing out this mistake; this was not meant to state 'AD'. However, we now include results for parental atopy which include and exclude eczema methods and results and Table III.

- Line (track changes manuscript) 219 Did you also investigate whether there was an interaction between EPE and parental atopy and peanut sensitization?

In line (track changes manuscript) 267-269 we stated that in the nested cases analysis 'There was no significant interaction between EPE and either infantile eczema, egg sensitization or FLG loss-of-function mutations on primary peanut sensitization (data not shown).'

We were not able to assess an interaction between EPE and parental atopy with peanut sensitization as the case control analysis was set up by matched for parental atopy.

Discussion

- In the light of my questions and comments regarding possible confounders and effect modifiers between the association of EPE and peanut sensitization, it would be necessary to comment on this in the discussion section of the manuscript.

We have included a discussion of the additional variables included in the analysis (eczema severity and allergic reactions to a specific food by age 8 years) in the discussion (lines (track manuscript) 353-359 and 372-375):

'Measures of eczema severity (extent of localisation, topical steroid use and persistent eczema) were associated with primary peanut sensitization on univariate analysis but were no longer associated on multivariate analysis. Eczema severity has previous been shown to be an independent risk factor for food sensitization in the EAT (Enquiring About Tolerance) study.[28] The EAT study used the SCORAD eczema severity scoring system; however, this could not be performed in the BAMSE study as the evaluations at 1,2,4 and 8 years were based on parental questionnaires. Tree nut and/or soya allergy by age 8 was also associated with primary peanut sensitization at age 8 years; co-existent peanut and tree-nut allergy has been shown

in numerous studies,[32, 33] whereas co-existent soya and peanut allergy is reportedly low.[34]'

We have also included a section in the discussion outlining the variables that we could not include which may be possible confounders (line (track changes manuscript) 380-386:.

'Certain potential confounders were not available such as parental peanut allergy or other parental food allergies, maternal peanut consumption during pregnancy and breast-feeding and infant peanut consumption. Therefore we are unable to assess whether infant peanut consumption could have protected the children against environmental exposure to peanut as has been shown in previous studies;[12, 32] however, when BAMSE was recruiting participants in 1996 the number of infants eating peanut was very low.'

- How does the amount of EPE in this birth cohort study correspond to other studies?

The median level of peanut protein in maternal bed-dust dust in the BAMSE cohort 4.07µg/gram [IQR 1.58, 11.86] was similar to that found in another the UK study by our group (median 4.19 IQR 0.54-24.89) (Brough et al 2013 JACI; 132(3):623-9).

The level of peanut in dust was higher in a US study (Brough et al. JACI 2015; 135(1):164-70) that measured peanut from the living room floor (median 39.1ug/g IQR 0.4-1.33). It would however be expected that peanut levels would be higher in America due to higher levels of peanut consumption. This information has been added to the discussion of the manuscript (line (track changes manuscript) 339-344).

- How do you explain the difference between the association of EPE with extract versus component sensitization?

We believe the difference between the association of EPE with extract versus component peanut sensitization supports the hypothesis that EPE increases the probability of developing clinically relevant peanut sensitization, and therefore that these children are more likely to develop peanut allergy.

In previous work, Brough et al. (2013 JACI) showed that peanut allergens in dust were able to activate basophils of peanut allergic individuals in an allergen specific manner. We therefore hypothesise that peanut levels in dust are also able to interact with dendritic cells and T cells in the development of clinically relevant peanut allergic sensitization.

- Line (track changes manuscript) 269 How does your data support this conclusion?

In line (track changes manuscript) 268-270 we stated: 'we did show on subgroup analysis that early EPE has more impact in children with certain risk factors for the development of peanut sensitization (such as parental atopy or egg sensitization) than children without these risk factors'. Our data support this conclusion because in our nested cases analysis, the association between EPE and peanut sensitization was stronger when comparing peanut sensitized cases versus high risk controls (matched for parental atopy). In the whole cohort analysis the association between EPE and peanut sensitization was stronger when comparing peanut sensitized children versus high risk controls (defined by egg sensitization).

We have amended the sentence in the manuscript to better clarify how our data supports this conclusion (line (track changes manuscript) 332-335): 'we did show on subgroup analysis that early EPE has more impact on the probability of developing peanut sensitization when compared against high risk children (defined by parental atopy in the nested cases analysis and egg sensitization in the whole cohort analysis) than in children without these risk factors'. However, we also state in the discussion that 'findings on case control analysis could not be replicated on whole cohort analysis, therefore these findings need to be confirmed in other population based cohorts.'

Minor points

- Throughout the article the abbreviation "protein allergen molecules (PAM)" is used while this is no common terminology for component resolved diagnostics or sIgE to protein components.
- Line (track changes manuscript) 100 "...(PAM) analysis of sIgE to the seed storage proteins...
- Line (track changes manuscript) 122 "... peanut PAM sensitized if sIgE to..."

Thank-you for raising this point. We have removed the term protein allergen molecule and PAM and have replaced this by stating sIgE to peanut protein allergen components or where we refer to Ara h 1,2 or 3 >0.35kU/l we state 'primary peanut sensitization'.

- Table E1: the percentage is now confusing why not present a column with missing data separately?

We have clarified that the percentage is the ratio of cases compared to the number of available data points. If the reviewer wishes we can also include a separate table with missing data as E2.

Reviewer: 2

Comments for Authors:
The paper explores an interesting concept- that early transcutaneous sensitization to peanut allergen is a risk factor for the development of subsequent peanut allergy. I found the paper hard to read with a number of grammatical mistakes and some paragraphs were not very clear. Importantly this includes the last two paragraphs of the Abstract: this is where the average reader makes the decision whether they will read on or move on.

Thank-you for highlighting this. We have made several amendments to the abstract following the comments from reviewers and we hope that this now reads more clearly. In particular we have simplified the conclusion so that it now reads as follows (line (track changes manuscript) 59-63): 'Higher levels of environmental exposure to peanut in the first few months of life appear to increase the probability of developing school age peanut sensitization in atopic children (based on egg sensitization and parental atopy).'

Much reliance is made that the filaggrin loss of function mutations chosen represent the majority of those found in this population but their prevalence appears very low- are other mutations perhaps present which means groups have been mis-classified?

The prevalence of FLG loss-of-function mutations is low as this is an unselected population based birth cohort rather than a high-risk eczema cohort. In European populations the two most prevalent mutations in the FLG gene are 2282del4 and the R501X; this has been widely replicated in many studies. Three FLG mutations analysed (2282del4, R501X and R2447X) had frequencies of 4%, 2% and 1% respectively in the BAMSE study which are similar to the frequencies found in a German cross-sectional material (Weidinger et al JACI Vol 121, No 5, 1203-1209, 2008). The mutation next in frequency (0.2%) is S3247X. It is reasonable to assume that the frequency for this mutation would be at a similar level in the German and Swedish cohorts. S3247X carriers in the BAMSE cohort would therefore have amounted to approximately 4 individuals. Therefore, we do not think that the additional information from these few individuals would change any of the conclusions in our study.

It would have been valuable to have measured transepidermal water loss in all infants or particularly the high atopy group.

We agree with the reviewer that measurement of transepidermal water loss during infancy would have been a valuable measure of skin barrier function; however, the BAMSE cohort children were recruited in 1996 and at this time the body of evidence linking TEWL to eczema was not yet available.

The study suffers from its design- the reviewer suspects the original hypothesis behind BAMSE did not include measurement of peanut allergen nor the early development of eczema.

The reviewer is correct in stating that the original study design for BAMSE did not include measurement of peanut allergen as peanut allergy was very rare in Sweden in 1996. The early development of eczema was; however, one of the original analyses for the BAMSE study.

It would surely have been preferable to measure peanut allergen in the infant's cot where they spend most of their early life rather than their mother's bed?

Unfortunately dust was not sampled from the infant's cot in the BAMSE study. The rationale for this was that most of the infants recruited to the study were first-borns thus the mattress would have been new. We have, however, shown in a previous publication that there is a high correlation between levels of peanut protein in the bed of the mother and infant-cot (correlation coefficient 0.844 (95% CI: 0.708-0.919), p<0.001) during the first year of life. (Brough et al. Distribution of peanut protein in the home environment. J Allergy Clin Immunol 2013;132:623-9).

Definition of eczema based on questionnaire is prone to major errors as has been seen in other epidemiological studies- to reach firm conclusions infants must be regularly examined prospectively.

The eczema questionnaires used at age 1 and 2 years in the BAMSE study were previously validated in the publication by Bohme M et al. (Atopic Dermatitis and Concomitant Disease Patterns in children up to two year of age. 2002;82:98-103).

Fifty consecutive children aged 0-4 years of age were seen in clinic and their parents were asked to fill in this questionnaire before meeting the doctor. The questionnaire contained, among other questions, the questions diagnostic for eczema from the main study questionnaires. The children were then examined by a Dermatologist to determine whether they had eczema according to the criteria from Hanifin JM and Rajka G. Diagnostic features of atopic dermatitis. Acta Derm Venereol 1980; Suppl 92:44-47. The sensitivity of the questionnaire assessment compared with the clinical diagnosis by the dermatologist was 92% and the specificity was 100%.This information has been included in the manuscript (line 171-172) and the reference from Bohme et al has also been included (ref 20).

There is as a result little information of eczema SEVERITY in infancy- which must surely be relevant to degree of sensitization if this hypothesis is correct (peanut sensitization occurs first through the skin).

Eczema severity (defined by extent of localisation of eczema, use of topical steroids and persistent eczema at age 8 years) was positively associated with peanut sensitization on univariate analysis, but was no longer significant on multivariate analysis. This is in contrast to other studies that have shown that eczema severity is an independent risk factor for sensitization; however these studies used SCORAD assessments which were not available in BAMSE.

We are not told of maternal allergic status- could it be allergen levels were lower in some homes because of maternal peanut allergy? Do we know of maternal peanut intake in pregnancy and perhaps more importantly whilst breast-feeding [I understand the majority of Swedish mothers choose to breast feed-] did those with atopic personal or infant history do so for longer?

The BAMSE cohort does not unfortunately have information on parental peanut allergy, parental food allergy or maternal peanut consumption during pregnancy or breastfeeding as it was not originally designed to assess peanut allergic outcomes. There is evidence that mothers breastfed marginally longer in BAMSE if there was one atopic parent vs no atopy (exclusive breastfeeding 79.3% if no parental atopy vs 80.8% if one parent atopic); if both parents were atopic this increased to 85.8% (Kull et al JACI 2004; 114:755-60 (Table 1)). Duration of breastfeeding (both exclusive and partial) has been included as a covariate in the analysis (Table I and III).

It is hardly surprising infants with eczema have a higher rate of sensitization- this has been repeatedly demonstrated but it has also been demonstrated such sensitization commonly does NOT translate into clinical allergy, something the authors have not sought to demonstrate. It has recently been demonstrated in an ingenious paper (Fukuie T et al (J Dermatol. 2016 Nov;43(11):1283-1292) that measures of sensitization improve as better eczema control is achieved. As the authors of this present study are aware, sensitization does NOT translate into allergy. The authors rely on high levels of specific IgE to components of peanut and argue this reflects a higher likelihood these children are also peanut allergic but a very recent paper has cast serious doubt on the reliability of levels of component-based [protein allergen molecules] specific IgE to predict severe allergy[van Veen et al. BMC Pediatrics (2016) 16:74].

We agree that the presence of IgE directed against peanut protein component allergens does not always translate into allergy; however, the premise of this study was to assess whether environmental peanut exposure is associated with peanut sensitization as the first step towards allergy. We would have liked to assess whether EPE was associated with OFC proven peanut allergy; however, this information was not available in BAMSE as the study was not designed to assess peanut allergic outcomes.

The study by van Veen et al. (BMC Pediatrics; 2016: 16:74) assesses the diagnostic performance of peanut SPT, peanut sIgE to whole extract and peanut sIgE to component allergens (Ara h 1,2 and 3) against oral food challenges to peanut. They found that peanut SPT and peanut sIgE against component allergens were significantly associated with a positive outcome on peanut oral food challenge ($p<0.0001$); however, they were not associated with the severity of reaction or eliciting dose on food challenge.

In this manuscript we did not assume that primary peanut sensitization (Ara h 1,2 or 3 >0.35kU/l) was linked to severe peanut allergy; only that it was more likely to predict clinical peanut allergy (regardless of the level of severity).

I am surprised the authors report allergic rhinitis at 8 years in these groups (which appears at least at first sight to have little to do with peanut sensitization or allergy but may have minor interest in understanding false-positive specific IgE to Ara-8) but

do not report how many children still suffer from eczema- a surrogate marker of their early eczema severity.

We did indeed include allergic rhinitis at 8 years as a marker for false positive IgE directed against Ara h 8. We have now included this information as a footnote of Table1. Eczema at age 8 years (OR 2.85, 95% CI: 1.69-4.81, p<0.0001) was associated with peanut sensitization at age 8 years on univariate analysis but was no longer associated on multivariate analysis.

The authors should discuss why cutaneous peanut exposure in British and Swedish children with early onset eczema is apparently associated with later peanut sensitization (and purportedly peanut allergy) whereas high levels of exposure in the same circumstances in Israel (where early infantile eczema is also very common and where peanut consumption is actually higher than either of those european countries) does not.

In Israel, infants are weaned onto a peanut containing food from 4 months of age which we believe is the reason why children in Israel do not have high levels of peanut allergy despite high household peanut consumption. This observation was published by (Du Toit G JACI 2010) and was the basis of the intervention for the LEAP study. The protective effect of infant peanut consumption against environmental peanut exposure was also shown in the paper by Fox et al. (J Allergy Clin Immunol 2009;123:417-23) where household peanut consumption during infancy increased the risk of developing peanut allergy, but not in children already consuming peanut during the first year of life. The Dual Allergen Exposure Hypothesis (Lack G JACI 2010) describes this balance of exposures during the first year of life where depending on whether the exposure to peanut is through the skin or gut, the immune system is then primed to develop allergy versus tolerance respectively.

We unfortunately do not have information on peanut consumption during infancy in the BAMSE study, thus we are unable to show the protective effect of oral consumption on preventing the development of peanut allergy. However peanut consumption in 1996 in Sweden was low. We have added the following text into the discussion (line (track changes manuscript) 380-386):

'Certain potential confounders were not available as the BAMSE study was not originally designed to evaluate risk factors for peanut allergy, such as parental

peanut or other food allergy, maternal peanut consumption during pregnancy and breast-feeding and infant peanut consumption. Therefore we are unable to assess whether infant peanut consumption could have protected the children against peanut sensitization as has been shown in previous studies (Fox et al. J Allergy Clin Immunol 2009;123:417-23, Du Toit G et al. J Allergy Clin Immunol. 2008 Nov;122(5):984-91); however, when BAMSE was recruiting participants in 1996 the number of infants eating peanut was very low.'

It is also disappointing studies where at risk infants are prospectively treated with emollient (to reduce skin exposure to allergens) have shown whilst eczema severity is reduced, allergic sensitization is not.

We agree that the study by Horimukai K et al. JACI 2014 did not show a reduction in sensitization to egg despite a 32% reduction in eczema; however, the IgE measurements for egg used in this study were not standard immunoCAPS therefore we are unsure as to validity of these measures. In addition, this was a small study thus with larger numbers an effect may be seen given that there was a higher egg sensitization rate in those children who developed eczema versus in children who did not develop eczema (odds ratio, 2.86; 95% CI, 1.22-6.73). We await the results of the Barrier Enhancement and Eczema Prevention (BEEP) study which will be assessing peanut sensitization after emollient application during infancy.

Environmental peanut exposure increases the risk of peanut sensitization in high risk children

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Word count: 3528

Short Title: Environmental peanut exposure and peanut sensitization in BAMSE

Abstract

Background: High household peanut consumption is associated with the development of peanut allergy, especially when peanut allergic cases are compared against atopic controls; thus environmental peanut exposure (EPE) may be a risk factor for peanut sensitization and allergy. In this study we explored the relationship between EPE and school-age peanut sensitization in a population based cohort.

Methods: Maternal bed-dust was collected postnatally and EPE was quantified using a polyclonal peanut ELISA. Peanut sensitization was assessed by specific IgE to peanut extract and sIgE to peanut protein component allergens Ara h 1, 2 or 3 ≥0.35kU/L (primary peanut sensitization). Initial nested case control analysis was performed comparing peanut sensitized cases against high-risk controls (matched for parental atopy) (n=411) using a conditional regression analysis. This was followed by whole cohort analysis (n=1878) comparing EPE against peanut sIgE sensitization at ages 4 and 8 years using Generalized Estimating Equations and against primary peanut sensitization at age 8 years using a logistic regression model. Finally, a subgroup analysis was performed comparing the impact of EPE in peanut sensitized versus egg-sensitized, peanut tolerant individuals using logistic regression analysis. Levels of EPE were compared between groups using the Mann-Whitney U test.

Results: In the nested case control analysis, a higher level of EPE around birth was associated with peanut specific IgE sensitization at age 4 years (OR=1.41, 95% CI:1.05-1.90), and primary peanut sensitization at age 8 years (OR=2.11, 95% CI:1.38-3.22) compared against high-risk controls. When the whole BAMSE cohort was assessed, EPE was no longer associated with peanut sensitization; however, on subgroup analysis EPE was associated with primary peanut sensitization when compared against egg-sensitized peanut-tolerant controls with an adjusted odds ratio of 1.44 per unit EPE (95% CI:1.06-1.94). There was no significant interaction between EPE and *FLG* loss-of-function mutations, egg sensitization at age 4 years, infantile eczema or parental atopy on peanut sensitization.

Conclusions: Higher levels of environmental exposure to peanut in the first few months of life appear to increase the probability of developing school age peanut sensitization in atopic children (based on egg sensitization and parental atopy).

Abstract word count: 343

Key words: BAMSE, egg sensitization, environmental peanut exposure, *FLG* mutation, infantile eczema peanut sensitization

Funding

The research was funded/supported by Action Medical Research (S/P/4529) and by the National Institute for Health Research (NIHR) Clinical Research Facility at Guy's & St Thomas' NHS Foundation Trust and NIHR Biomedical Research Centre based at Guy's and St Thomas' NHS Foundation Trust and King's College London. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health. The BAMSE study was funded by the Swedish Research Council, the Stockholm County Council, the Swedish Research Council for Health, Working life, and Welfare the Swedish Asthma and Allergy Foundation, the Swedish Heart and Lung Foundation, and the Gillbergska Foundation.

Abbreviations

AIC: Akaike information criterion

Ara h: Arachis hypogaea

BAMSE: Children/'Barn' Allergy Milieu Stockholm Epidemiological project

CI: confidence intervals

EPE: environmental peanut exposure

FLG: gene encoding the protein filaggrin

GEE: Generalized Estimating Equations

ISAAC: International Study of Asthma and Allergies in Childhood

IQR: Interquartile range

QIC**:** Quasi Likelihood Independence models criterion

Introduction

Peanut allergy has increased,[1, 2] is a leading cause of anaphylaxis in food allergy[3] and has a significant impact on quality of life for the child and their family.[4, 5] Most children react on first known oral exposure to peanut; thus sensitization must be occurring earlier.[6, 7] Understanding the way children become sensitized to peanut is therefore imperative in order to prevent this condition. Observational[8] and animal work[9, 10] suggests that epicutaneous peanut exposure and early onset severe eczema may play an important role in peanut sensitization. Epicutaneous exposure may be the route that peanut levels in dust sensitize a child in early life, particularly if the skin barrier is broken through eczema or specific genetic mutations associated with skin barrier dysfunction (e.g. filaggrin (*FLG)* mutations or eczema). We have recently shown in the Manchester Asthma and Allergy Study (MAAS) and Consortium of Food Allergy Research (CoFAR) cohort that *FLG* loss-of-function mutations and eczema severity respectively can increase the impact of early EPE on peanut sensitization and allergy.[11, 12]

A dose response relationship has also been demonstrated between household peanut consumption (used as an indirect marker for environmental peanut exposure: EPE) and the risk of developing peanut allergy in young children.[13] Fox et al. compared household peanut consumption in peanut allergic individuals against children who were at high risk of developing peanut allergy (due to the presence of egg allergy) but had not developed peanut allergy. Household peanut consumption was ten times higher in households with infants with peanut allergy versus children with egg allergy without peanut allergy but was only 3 times higher in non atopic controls, highlighting that household peanut consumption was more likely to be associated with peanut allergy in high risk children.[13] Household peanut consumption and peanut sensitization has also been associated in other studies.[14] Peanut antigen in the infant's bed-dust and play-area-dust is highly positively correlated with household peanut consumption and stimulates basophils from peanut allergic children, thus has the potential to also sensitize individuals.[15]

Peanut sensitization (specific IgE≥0.35kU/L) is present in up to 10% of children and protein allergen molecule analysis of the seed storage proteins in peanut (Ara h 1, 2 and 3) has been shown to improve the specificity and sensitivity of diagnosing peanut allergy.[16, 17] In BAMSE, children with IgE reactivity to Ara h 1, 2 or 3 at age 8 years (but not Ara h 8) reported peanut allergic symptoms in 87% of cases, whereas children with IgE reactivity exclusively to Ara h 8 reported peanut allergic symptoms in only 17% of cases which were also milder.[18] Sweden has high levels of birch pollen sensitization which explains the Bet v 1 in-vitro cross-reactivity with peanut Ara h 8;[18-20] thus, in this study we differentiated between primary peanut sensitization (IgE reactivity to Ara h 1, 2 or 3) and pollen cross-reactivity (IgE reactivity to Ara h 8).

This study aimed to assess whether EPE (as defined by peanut protein levels in household dust) is a risk factor for the development of school-age peanut sensitization. The analysis was done in two steps for financial and logistical reasons; firstly among high risk children (with parental atopy matched to peanut sensitised cases), in a nested cases analysis and secondly among the whole studied population (a cohort analysis). Given previous findings we also wanted to ascertain the modifying effects of *FLG* loss-of-function mutations, infantile eczema and preceding egg sensitization.

Methods

The BAMSE study is an unselected Swedish population birth cohort. The design and methodology are described elsewhere.[21] In brief 4089 unselected newborn children were recruited from 1994-1996 and have been evaluated for various health conditions over time. No intervention was performed. The BAMSE study obtained ethical approval for environmental sampling of dust.

Demographics

Serum specific IgE to peanut (ImmunoCAP system, ThermoFisher, Sweden) was measured at 4 and 8 years and children were defined as 'peanut sIgE sensitized' if peanut specific IgE was ≥0.35kU/L. Peanut protein component allergens Ara h 1,2,3,8 and 9 were assessed at 8 years (ImmunoCAP system, ThermoFisher, Sweden) in all children sensitized to peanut at age 8 years, irrespective of sensitization to birch. In addition, those children who were peanut sensitized at age 4 years but lost their sensitization at age 8 were also analyzed for these allergens. All other children with negative peanut sIgE at age 8 years were assumed to have negative component allergens to peanut. Children were considered to have primary peanut sensitization if Ara h 1, 2 or 3 was ≥0.35kU/L and no primary peanut sensitization if Ara h 1, 2 and 3 were <0.35kU/L or peanut sIgE was <0.35kU/L at 8 years (where no sIgE peanut protein component allergens were available*).* Twenty-four (1.3%) children with positive peanut specific IgE at 8 years of age but with no available serum for peanut protein component allergen analysis were excluded from this analysis. Egg sensitization was defined as egg specific IgE ≥0.35kU/L at 4 years of age. Previous allergic reactions to a food including itching in the mouth, nose/eye problems, trouble breathing, vomiting or diarrhoea, eczema or nettle rash was used from parental questionnaires at age 8 years.

Parental atopy was defined as a doctor's diagnosis of asthma and prescription of asthma medication and/or a doctor's diagnosis of hay fever in combination with furred pets- and/or pollen allergy at the time of questionnaire. This definition of parental atopy was selected rather than the same definition with inclusion of a doctor's diagnosis of eczema (excluding contact dermatitis); this was because the children of parents with atopy including eczema were less atopic (lower infantile

eczema) and parental atopy including eczema was not associated with the outcome of interest (primary peanut sensitization at 8 years of age); this is probably because adult eczema is often non-atopic. Genotyping was performed for *FLG* mutations common in Scandinavia by using TaqMan allelic discrimination assays for R501X and R2447X and matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry for 2282del4. Children with a *FLG* mutation in any of these positions were classified as having a *FLG* loss-of function mutation.

A reported history of infantile eczema was assessed by a validated eczema questionnaire with sensitivity of 92% and specificity of 100%.[22] Eczema was defined as dry skin, itchy rashes for 2 weeks or more and age specific localization (face or arms/legs extension surfaces or arms/legs flexures or wrists/ankles flexures) and/or doctor's diagnosis of eczema after 3 months and up to 1 year of age. Severity of eczema was assessed from questionnaires using three different measures:

1) Extent of localization of eczema (up to 10 areas) at or before their one year assessment

2) Topical steroids used as treatment for an itchy rash in the last year at their two year assessment (not available at their one year assessment)

3) Persistent eczema at age 8 years (using the same definition as above).

Ethnicity was based on where the parents were born. Allergic rhinitis at 4 and 8 years was assessed using International Study of Asthma and Allergies in Childhood (ISAAC) validated questionnaires, and was defined as persistent rhinitis without a common cold the last 12 months before 4 and 8 years respectively.[23]

Dust collection methodology

Dust samples were collected at a median of 2 months of age from the mother's mattress. The mother's mattress was vacuumed for 2.5 minutes with a small disposable filter bag (Allergy Control Products Inc. Ridgefield CT, USA) inserted in the front hose of the vacuum. The dust containing filter bags were sealed in plastic bags and stored at -20ºC. Dust samples were sieved and fine dust

was weighed and extracted in proportional volumes of extraction solution as previously described.[24] Peanut protein in dust was determined using the Veratox polyclonal ELISA against whole peanut protein (Neogen Corporation, Lansing, MI, USA). The lower limit of quantitation (LLQ) of the assay was defined as 100ng/ml and samples below this value were defined as 50ng/ml (LLQ/2).[25] Results were converted from ng/ml into µg peanut protein/gram dust. Due to batch to batch variability of the Veratox ELISA results were batch corrected prior to being entered into the final statistical analysis.

Statistical analysis

Data was entered into an SPSS (SPSS 19.0; SPSS Inc, Chicago, IL, USA) and STATA spreadsheet (Timberlake Consultants Ltd London, UK) for the purpose of analysis. Peanut protein levels in dust (µg/gram) underwent natural log transformation. Initial nested case control analysis was performed with peanut sIgE sensitized cases at 4 years of age matched for gender and parental atopy with a 2:1 control (n=274) to case (n=137) matching. Children with primary peanut sensitization defined by Ara h 1, 2 or 3 ≥0.35kU/L at 8 years of age were matched against controls at age 8 years with the same sex and level of parental atopy with a 2:1 ratio of controls (n=130) to cases (n=65). Given that these controls were matched for the higher levels of parental atopy in the primary peanut sensitized cases these controls were deemed to be high-risk as the primary peanut sensitized cases had higher levels of parental atopy than the population overall (46.7% vs. 32.6%). Conditional logistic regression (incorporating matching) was performed for the case control analysis using robust standard error for peanut sIgE sensitization at 4 years and primary peanut sensitization at age 8 years.

Subsequently analysis was performed for the relationship between early EPE and peanut sensitization in all children from the BAMSE cohort with available postnatal maternal bed-dust and *FLG* genotyping (n=1878). Logistic regression analysis was performed for primary peanut sensitization at 8 years of age. Factors associated with peanut sIgE sensitization were assessed using Generalized Estimating Equations (GEE) with an exchangeable working correlation matrix to

account for repeated measures within individuals at 4 and 8 years. Univariate followed by multivariate regression analysis was performed including EPE plus other covariates significantly associated with peanut sensitization ($P \leq .05$) which also improved the quality of fit of the multivariate model using the Akaike information criterion (AIC) for logistic regression and the Quasi Likelihood Independence models criterion (QIC) for GEE analyses.

Where the *FLG* mutation covariate was entered into the statistical model, participants with missing ethnicity data (35/1878=1.9%) and non-caucasians (165/1842=8.8%) were excluded as distinct *FLG* loss-of-function mutations are present in different populations and the *FLG* loss-of-function mutations assessed in this study have only been associated with eczema in Caucasian European populations [26, 27] Peanut levels in dust (µg/g) were compared between groups using the Mann-Whitney U test. Statistical significance was assessed at $P < .05$.

Results

Details of demographics and clinical characteristics included on this study are described in Table EI. Median peanut protein [IQR] in dust was 4.07µg/gram [1.58, 11.86]. Peanut extract sIgE sensitization was 6.6% at 4 years (n=103/1572) and 8.6% at 8 years (n=161/1876). Primary peanut sensitization at 8 years was 4.1% (n=75/1854), of which 74/75 (99%) had Ara h 2 ≥0.35kU/l. We first performed a nested case control analysis, and based on positive findings for this subsequently proceeded to then analyse the whole BAMSE cohort.

Nested case control analysis for peanut sIgE and primary peanut sensitization

We compared peanut sensitized children against children without peanut sensitization matched for gender and parental atopy as described in the statistical methods; EPE was a risk factor for both peanut extract sIgE sensitization at 4 years and primary peanut sensitization at 8 years with a 23% and 29% increased probability of developing peanut sensitization per natural log (*ln*) unit increase in EPE respectively (Table I). In children who were peanut extract sIgE sensitized but not sensitized to peanut protein component allergens Ara h 1, 2 and/or 3, there was only borderline significance towards an association between EPE and peanut sensitization (OR=1.20, 95% CI: 0.97-1.48).

Differential relationship between EPE and primary peanut sensitization and non-clinically significant peanut sensitization is displayed in Figure 1. Median peanut protein in dust was higher in peanut extract sIgE sensitized children (3.39 µg/g, IQR 1.41-11.01, n=137) than non-peanut extract sIgE sensitized controls (2.28µg/g, IQR 0.88-5.14, n=274) (*P*<.01); and higher in primary peanut sensitized children (4.64µg/g, IQR 1.58-12.77, n=65) than in control children that were not sensitized to Ara h 1,2 or 3 (2.24µg/g, IQR 0.88-4.74, n=130) (*P*<.01).

On multivariate conditional logistic regression analysis, EPE was significantly associated with peanut specific IgE and primary peanut sensitization with a 1.41- and 2.1-fold increased probability

of developing peanut sensitization per unit increase EPE respectively (Table II). Infantile eczema and egg sensitization were both significantly associated with sensitization to peanut extract and sensitization to peanut protein component allergens Ara h 1, 2 and/or 3.(Table II). *FLG* loss-of function mutations increased the probability of developing peanut specific IgE sensitization 3.78-fold and primary peanut sensitization 7.33-fold. Allergic rhinitis at age 4 and 8 years was associated with both the probability of developing peanut specific IgE sensitization at age 4 years and primary peanut sensitization at age 8 years in the multivariate analysis. The number of biological siblings, duration of exclusive and total breastfeeding and maternal age at baseline were not associated with peanut sensitization. There was no significant interaction between EPE and either infantile eczema, egg sensitization or *FLG* loss-of-function mutations on primary peanut sensitization (data not shown).

Whole cohort analysis for peanut extract sIgE sensitization and primary peanut sensitization

In a second step following positive findings obtained on case control analysis, we assessed the relationship between early EPE and peanut sensitization in all children from the BAMSE cohort with available postnatal maternal bed-dust and *FLG* genotyping (n=1878). On both univariate (Table III) and multivariate analysis (Table IV) infantile eczema, egg sensitization at age 4 years, tree nut and soya allergy at age 8 years, *FLG* loss-of function mutation and allergic rhinitis at age 8 years were associated with the probability of peanut sIgE sensitization at age 4 and 8 years. For primary peanut sensitization at age 8 significant associations were also seen on univariate (Table III) and multivariate analysis (Table IV) for infantile eczema, egg sensitization, tree nut and soya allergy at age 8 years and allergic rhinitis at age 8 years. Although measures of eczema severity and several other food allergies at age 8 years were significantly association with peanut sensitization on univariate analysis, these variables were no longer significantly associated on multivariate analysis. EPE was not associated with peanut sIgE sensitization or primary peanut sensitization. There was no interaction between EPE and *FLG* mutations, infantile eczema, egg sensitization, allergic reaction to tree nut or soya by age 8 years, or parental atopy on peanut sensitization.

Subgroup analysis of primary peanut sensitization against egg sensitized non peanut allergic individuals

On subgroup analysis we compared primary peanut sensitized children (n=75) against children who were sensitized to egg (sIgE≥0.35kU/L at age 4 years) but did not go on to have primary peanut sensitization at age 8 years (n=59). EPE increased the probability of primary peanut sensitization on both univariate (OR=1.33 95% CI:1.01-1.74, *P*<.05, n=134) (Table V) and multivariate analysis (OR=1.44, 95% CI:1.06-1.94, P0.018, n=133, AIC: 167) adjusting for infantile eczema and allergic reaction to soya by age 8 years. Peanut levels in household dust were also significantly higher in primary peanut sensitized children (median 4.79µg/g, IQR 1.63-12.00) versus egg sensitized, non-primary peanut sensitized children (median 2.35µg/g, IQR 1.23-6.15) (*P*=.03). We assessed the impact of EPE on peanut sensitization when using other high-risk groups of children who were not peanut sensitized at age 8 years (*FLG* mutations, infantile eczema, soya allergy and parental atopy) and there was no significant association.

Discussion

In the prospective birth cohort BAMSE, there was an exposure-response relationship between EPE (quantified using peanut protein levels from maternal mattress-dust postnatally) and primary peanut sensitization (Ara h 1, 2 or 3 ≥0.35kU/L) among high risk children (in a nested cases analysis). When all children in the whole studied population were assessed, there was no association between EPE and peanut sensitization; however, on subgroup analysis there was an exposure-response relationship between EPE and primary peanut sensitization when compared against high risk egg sensitized children. Fox et al. also showed that household peanut consumption during the first year of life (as an indirect marker of EPE) was higher in peanut allergic cases than in egg allergic controls.[13] In the nested cases analysis the exposure-response relationship was stronger between EPE and primary peanut sensitization (Ara h 1,2 or 3≥0.35kU/L) than between EPE and non-clinically relevant peanut sensitization (peanut sIgE ≥0.35kU/L but negative results for Ara h 1,2 or 3<0.35kU/L) (Figure 1). This supports the concept that EPE increases the probability of developing clinically relevant peanut sensitization which is more likely to develop into peanut allergy rather than non-specific peanut sensitization.

In other cohorts we have shown a synergistic effect between EPE and markers of an impaired skin barrier (infantile eczema and *FLG* loss-of-function mutations) on peanut sensitization and allergy.[11, 12] Although in BAMSE we did not find significant interactions between EPE and *FLG* mutations or infantile eczema on peanut sensitization, we did show on subgroup analysis that early EPE has more impact on the probability of developing peanut sensitization when compared against high risk children (defined by parental atopy in the nested cases analysis and egg sensitization in the whole cohort analysis) than in children without these risk factors. The BAMSE cohort is relatively non-atopic, being from the general population in Sweden, which may have contributed to lower strength of associations between EPE and atopic markers. The median level of peanut protein in maternal bed-dust dust in the BAMSE cohort was 4.07µg/gram [IQR 1.58, 11.86], similar to that found in another the UK study by our group (median 4.19 IQR 0.54-24.89). [24] The level of peanut in dust was higher in a US study that measured peanut from the living room floor (median 39.1ug/g IQR 0.4-

1.33). [10] It would; however, be expected that peanut levels would be higher in America due to higher levels of peanut consumption.

Infantile eczema was one of the most important risk factors for peanut sensitization in BAMSE; this association has been cited in numerous studies.[8, 28] Measures of eczema severity (extent of localisation, topical steroid use and persistent eczema) were associated with peanut sensitization on univariate analysis but were no longer associated on multivariate analysis. Eczema severity has previous been shown to be an independent risk factor for food sensitization in the EAT (Enquiring About Tolerance) study.[29] The EAT study used the SCORAD eczema severity scoring system; however, this could not be performed in the BAMSE study as the evaluations at 1, 2, 4 and 8 years were based on parental questionnaires.

Egg sensitization was highly associated with peanut sensitization; which has previously been shown in numerous cohort studies;[11, 12, 30] it may be that egg sensitization shows a predisposition to mount allergic antibodies to other allergens as egg allergy is highly associated with the development of other food allergies, asthma and aeroallergen sensitization.[31, 32] Observational studies have shown that in children with severe, early onset eczema, up to 50% are sensitized to egg;[28] thus egg sensitization may be an additional marker of eczema severity. *FLG* loss-of-function mutations were less important than infantile eczema in predicting the development of peanut sensitization, and *FLG* mutations became more important if infantile eczema was not included in the regression model. Previous studies have found that children that carry a *FLG* mutation have an increased risk of peanut allergy even after adjusting for eczema.[33] In this study eczema seemed to be the overriding factor for peanut sensitization; however, it should be noted that *FLG* mutations were quite low in BAMSE (7.14%) in comparison with other population-based cohorts.[26, 33, 34] Tree nut and/or soya allergy by age 8 was also associated with primary peanut sensitization at age 8 years; co-existent peanut and tree-nut allergy has been shown in numerous studies,[35, 36] whereas co-existent soya and peanut allergy is reportedly low.[37]

Strength of the present study includes the population based design and relatively high number of study participants. Limitations of this study included the lack of diagnostic food challenges to determine peanut allergy as the BAMSE study was not originally designed to evaluate peanut allergic outcomes; however, primary peanut sensitization has been shown to be a much more accurate predictor of clinically confirmed peanut allergy than peanut specific IgE.[38, 39] Certain potential confounders were not available such as parental peanut allergy or other parental food allergies, maternal peanut consumption during pregnancy and breast-feeding and infant peanut consumption. Therefore we are unable to assess whether infant peanut consumption could have protected the children against environmental exposure to peanut as has been shown in previous studies;[13, 35] however, when BAMSE was recruiting participants in 1996 the number of infants eating peanut was very low. Caucasian ethnicity was based on place of parental birth rather than self-reported ethnicity; however, when the BAMSE study was recruiting participants 1994-1996, most parents born in Sweden or Northern Europe would have been of Caucasian descent.[40] Findings on case control analysis could not be replicated on whole cohort analysis; therefore, these findings need to be confirmed in other population based cohorts.

Conclusion

In summary, although EPE did not increase the risk of peanut sensitization in children who did not already have atopic risk factors, the findings from the BAMSE cohort support the hypothesis that in specific high risk groups, peanut levels in dust around the time of birth may pose a risk for later peanut sensitization. Further prospective studies are needed to confirm these findings in other populations.

Acknowledgement

We wish to thanks Dr Alick Stephens for his assistance in the BAMSE dust analysis. The authors thank all children and their parents in the BAMSE cohort

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Table EI: Demographics of whole studied BAMSE population with available dust and *FLG* genotyping (n=1878)

	Number of cases	No. data points	Percentage of cases / no. of available data points
Peanut specific IgE ≥ 0.35kU/L at 4 years	103	1572	6.55%
Peanut specific IgE ≥ 0.35kU/L at 8 years	161	1876	8.58%
Ara h 1, 2 or 3 ≥0.35kU/L at 8 years vs. Ara h 1, 2 or 3 <0.35kU/L or peanut sIgE <0.35kU/L (excluding peanut sIgE ≥0.35kU/L with no components or missing data) at 8 yrs	75	1854	4.05%
History of infantile atopic dermatitis	313	1840	17.01%
Topical steroid use between age 1-2 years	534	1842	29.0%
Higher extent of localisation of eczema (>5 areas vs. <5 areas or no eczema)	49	1841	2.7%
Persistent eczema at age 8 years	321	1828	17.6%
At least one *FLG* mutation (R501X, 2282del or R2447x)	134	1878	7.14%
Allergic rhinitis (ISAAC definition) (age 4 years)	215	1828	11.76%
Allergic rhinitis (ISAAC definition) (age 8 years)	288	1865	15.44%
Egg sIgE≥ 0.35IU/ml at 4 years of age	82	1575	5.21%
Reported allergic reaction to any food 8 years	349	1869	18.7%
Reported allergic reaction to peanut by 8 years	120	1869	6.4%
Reported allergic reaction to milk by 8 years	53	1869	2.8%
Reported allergic reaction to egg by 8 years	36	1869	1.9%
Reported allergic reaction to fish by 8 years	12	1869	0.6%
Reported allergic reaction to shellfish 8 years	17	1869	0.9%
Reported allergic reaction to wheat by 8 years	9	1869	0.5%
Reported allergic reaction to soya by 8 years	21	1869	1.1%
Reported allergic reaction to apple by 8 years	83	1869	4.4%
Reported allergic reaction to peach by 8 years	44	1869	2.4%
Reported allergic reaction to kiwi by 8 years	92	1869	4.9%
Reported allergic reaction to avocado 8 years	14	1869	0.7%

Reported allergic reaction to banana by 8 years	17	1869	0.9%
Reported allergic reaction to carrot by 8 years	53	1869	2.8%
Reported allergic reaction to tree nuts 8 years	105	1869	5.6%
Male gender	973	1871	52.00%
Caucasian ethnicity	1677	1842	91.04%
Biological siblings	1695	1870	90.64%
Parental atopy – (asthma/AR/eczema)	838	1858	45.10%
Parental atopy – (asthma/AR)	605	1856	32.60%
Maternal age (mean, SD)	30.9 (SD 4.49)	1871	N/A
Peanut protein in maternal bed-dust (µg/g): median [IQR]	4.07 [1.58-11.86]	1878	N/A
Exclusive breast-feeding duration (months): mean (SD)	5.19 (2.43)	1838	N/A
Total breast-feeding duration (months): mean (SD)	8.73 (3.31)	1825	N/A

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Table I: Univariate conditional logistic regression incorporating matching for peanut sensitized cases versus non-sensitized controls matched for gender and parental atopy. Peanut sIgE sensitization (≥0.35kU/L) at age 4 years and primary peanut sensitization (Ara h 1, 2 or 3 ≥0.35kU/L) at age 8 years.

	Peanut sIgE sensitized at age 4 years (n=411)			Peanut PAM sensitized at age 8 years (n=195)		
	OR	95% CI	*P* value	OR	95% CI	*P*-value
EPE (µg/gram ln transformed)	1.23	1.06-1.43	**<.01**	1.29	1.04-1.61	**.02**
No. of biological siblings	1.38	0.63-3.03	.42	2.50	0.77-8.16	.13
≥1 *FLG* loss-of-function mutation (excluding non-caucasians)	2.67	1.18-6.04	**.02**	3.00	0.84-10.75	.09
History of infantile eczema	7.35	4.20-12.83	**<.001**	6.39	2.95-13.83	**<.001**
Egg sIgE sensitization: 4 years	18.91	7.50-47.66	**<.001**	12.85	3.78-43.67	**<.001**
*Allergic rhinitis: 4 years (ISAAC)	3.44	1.90-6.23	**<.001**	2.53	1.04-6.18	**.04**
*Allergic rhinitis: 8 years (ISAAC)	11.44	5.68-23.07	**<.001**	12.97	4.54-37.05	**<.001**
Exclusively breastfed (months)	0.95	0.88-1.03	.22	0.98	0.87-1.11	.77
Breastfed (excl/partial) (months)	0.99	0.93-1.05	.74	1.01	0.93-1.11	.76
Maternal age at baseline (in years)	0.99	0.94-1.04	.65	1.05	0.99-1.12	.12

*Allergic rhinitis was included to assess whether false positive results for peanut sensitization were being found due to cross-reactivity with grass or tree pollens.

Table II: Multivariate conditional logistic regression incorporating matching for peanut sensitized cases versus non-sensitized controls matched for gender and parental atopy. Peanut sIgE sensitization (≥0.35kU/L) at age 4 years and primary peanut sensitization (Ara h 1, 2 or 3 ≥0.35kU/L) at age 8 years. Non-caucasians were excluded due to inclusion of *FLG* mutations as a covariate.

	Peanut sIgE sensitized at age 4 years (excluding non-caucasians) (n=237) *AIC: 90.78			Primary peanut sensitization at age 8 years (excluding non-caucasians) (n=143) *AIC: 49.19		
	OR	95% CI	*P* Value	OR	95% CI	*P* Value
EPE (µg/gram ln transformed)	1.41	1.05-1.90	**.02**	2.11	1.38-3.22	**.001**
≥1 *FLG* loss-of-function mutation	3.78	0.93-15.36	.06	7.33	1.21-44.21	**.03**
History of infantile eczema	2.53	0.98-6.51	**.05**	3.93	1.03-15.00	**.05**
Egg sIgE sensitization: 4 years	28.00	5.24-149.39	**<.001**	39.9	1.47-1081.68	**.03**
**Allergic rhinitis: 8 years (ISAAC)	12.57	4.164-37.98	**<.001**	28.93	3.77-221.61	**<.001**

*Smaller AIC is better. **Allergic rhinitis at age 8 years was selected as it improved the fit of the model (AIC better than allergic rhinitis at age 4 years).

Table III: Univariate analysis of peanut specific IgE sensitization aged 4 and 8 years using GEE to account for repeated measures within individuals at 4 and 8 years and logistic regression for primary peanut sensitization at age 8 years in the whole BAMSE cohort (n=1878).

	GEE for peanut sIgE Univariate peanut sIgE sensitization at 4 +8 years			Univariate primary peanut sensitization at age 8 years		
	OR	95% CI	*P* Value	OR	95% CI	*P* Value
EPE (μg/gram ln transformed)	0.95	0.86-1.06	0.39	1.01	0.86-1.19	.89
Gender	1.14	0.83-1.56	0.43	1.41	0.88-2.26	.15
Full older siblings	1.08	0.63-1.85	0.77	0.86	0.40-1.81	0.68
≥1 *FLG* loss-of-function mutations	1.84	1.10-3.05	**0.02**	1.84	0.89-3.77	**0.10**
Questionaire based history of infantile eczema	6.49	4.66-9.02	**<.001**	7.94	4.91-12.83	**<.001**
Localisation of infantile eczema (continuous 1-10 areas/per unit)	1.48	1.37-1.59	**<.001**	1.53	1.40-1.68	**<.001**
Localisation of infantile eczema (<5 areas vs. 5+ areas)	7.69	4.26-13.55	**<.001**	10.4	5.24-20.65	**<.001**
Application of topical steroids for itchy rash (1-2 yrs)	3.24	2.35-4.46	**<.001**	3.95	2.46-6.34	**<.001**
Persistent eczema at age 8 years	3.87	2.76-5.44	**<.001**	2.85	1.69-4.81	**<.001**
Egg sIgE sensitization at 4 years	13.58	8.55-21.58	**<.001**	11.04	6.13-19.90	**<.001**
Allergic reaction to milk by 8 years	5.68	3.12-10.34	**<.001**	4.24	1.84-9.77	**0.001**
Allergic reaction to egg by 8 years	26.81	13.98-51.4	**<.001**	16.67	7.81-35.59	**<.001**
Allergic reaction to fish by 8 years	17.25	5.41-54.96	**<.001**	9.18	2.39-35.32	**0.001**
Allergic reaction to shellfish 8 years	15.59	6.07-40.02	**<.001**	3.70	0.82-16.71	0.09
Allergic reaction to wheat by 8 years	9.55	2.53-36.02	**0.001**	8.06	1.60-40.59	**0.01**
Allergic reaction to soya by 8 years	48.75	17.61-134.97	**<.001**	41.95	16.56-106.24	**<.001**

Allergic reaction to apple by 8 years	12.54	8.16-19.27	**<.001**	5.93	3.15-11.13	**<.001**
Allergic reaction to peach by 8 years	14.37	8.15-25.34	**<.001**	6.49	2.88-14.61	**<.001**
Allergic reaction to kiwi by 8 years	5.95	3.80-9.31	**<.001**	4.25	2.20-8.20	**<.001**
Allergic reaction to avocado 8 years	12.27	4.61-32.66	**<.001**	4.38	0.95-20.13	**0.06**
Allergic reaction to banana 8 years	12.19	5.07-29.30	**<.001**	3.44	0.77-15.40	0.11
Allergic reaction to carrot by 8 years	17.84	10.6-30.05	**<.001**	7.83	3.83-16.03	**<.001**
Allergic reaction to tree nuts 8 years	21.93	14.45-33.28	**<.001**	10.29	12.08-34.05	**<.001**
Allergic rhinitis aged 4 years	2.82	1.92-4.13	**<.001**	2.16	1.22-3.83	**0.008**
Allergic rhinitis aged 8 years	8.05	5.78-11.22	**<.001**	5.87	3.66-9.42	**<.001**
Parental atopy (asthma and/or AR)	1.77	1.28-2.44	**<.001**	1.77	1.10-2.83	**0.018**
Parental atopy (asthma, AR,eczema)	1.44	1.05-1.98	**0.02**	1.50	0.95-2.39	0.09
Non-caucasian ethnicity	1.58	0.97-2.57	0.07	1.09	0.49-2.43	0.79
Exclusively breastfed in months	0.98	0.94-1.06	0.92	0.97	0.88-1.06	0.49
Breastfed (exclusive/partial) in mo	0.99	0.94-1.03	0.57	0.97	0.91-1.04	0.43
Maternal age at baseline (in years)	1.02	0.98-1.05	0.44	1.02	0.97-1.07	0.53

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Table IV: Multivariate analysis of peanut specific IgE sensitization aged 4 and 8 years using generalised estimating equations (GEE) to account for repeated measures within individuals at 4 and 8 years and logistic regression analysis for primary peanut sensitization at age 8 years in the BAMSE cohort, Goodness of fit assessed by QIC and AIC.

	Multivariate peanut sIgE sensitization (excluding non-caucasians) (1367) QIC:935			Multivariate primary peanut sensitization (n=1521) AIC:386		
	OR	95% CI	*P* Value	OR	95% CI	*P* Value
EPE (µg/gram ln transformed)	0.94	0.80-1.11	.45	1.08	0.87-1.34	.48
≥1 *FLG* loss-of-function mutations	2.52	1.24-5.10	**0.01**		ns*	
History of infantile eczema	2.75	1.67-4.48	**<.001**	4.02	2.14-7.54	**<.001**
Tree nut allergic reaction by age 8 years	9.30	4.94-17.53	**<.001**	4.80	2.38-9.66	**<.001**
Soya allergic reaction by age 8 years	18.21	4.75-69.80	**<.001**	2.54	3.85-42.07	**<.001**
Egg sIgE sensitization at 4 years	7.66	3.92-14.95	**<.001**	4.24	1.98-9.09	**<.001**
Allergic rhinitis aged 8 years (ISAAC)	4.02	2.49-6.52	**<.001**	2.01	1.05-3.83	**0.034**

*ns: Not significant on multivariate analysis therefore not included in final multivariate analysis.

Table V: Univariate analysis of factors associated with primary peanut sensitization (at age 8 years) versus non primary peanut sensitization (at age 8 years) in high risk children with preceding egg sensitization (at age 4 years) (n=134).

	Primary peanut sensitization vs. non-peanut sensitized egg sensitized controls		
	OR	95% CI	*P* Value
EPE (µg/gram ln transformed)	1.33	1.01-1.74	**<0.05**
Gender	1.90	0.95-3.80	0.07
Full older siblings	0.61	0.17-2.13	0.44
≥1 *FLG* loss-of-function mutations (excluding non-caucasians)	1.09	0.33-3.56	0.89
History of infantile eczema	2.51	1.24-5.08	**0.01**
Localisation of infantile eczema (continuous 1-10 areas/per unit)	1.15	1.01-1.32	**0.04**
Localisation of infantile eczema (<5 areas vs. 5+ areas)	1.36	0.52-3.53	0.53
Application of topical steroids for itchy rash (1-2 yrs)	1.40	0.70-2.80	0.34
Persistent eczema at age 8 years	2.32	1.09-4.90	**0.03**
Allergic reaction to milk by 8 years	1.42	0.39-5.09	0.59
Allergic reaction to fish by 8 years	0.78	0.15-4.00	0.78
Allergic reaction to shellfish 8 years	0.30	0.06-1.58	0.16
Allergic reaction to wheat by 8 years	1.59	0.14-17.96	0.71
Allergic reaction to soya by 8 years	11.05	1.39-87.63	**0.02**
Allergic reaction to apple by 8 years	1.71	0.64-4.52	0.29
Allergic reaction to peach by 8 years	1.29	0.40-4.17	0.67
Allergic reaction to kiwi by 8 years	1.42	0.52-3.85	0.50
Allergic reaction to avocado 8 years	0.78	0.11-5.71	0.81

Allergic reaction to banana 8 years	0.38	0.07-2.13	0.27
Allergic reaction to carrot by 8 years	2.36	0.71-7.85	0.16
Allergic reaction to tree nuts 8 years	3.43	1.54-7.62	**0.002**
Allergic rhinitis aged 4 years (ISAAC)	0.87	0.38-1.96	0.73
Allergic rhinitis aged 8 years (ISAAC)	2.65	1.26-5.56	**0.01**
Parental atopy (asthma/ and /or hay-fever)	1.34	0.67-2.65	0.41
Non-caucasian ethnicity	2.55	0.93-6.98	0.07
Exclusively breastfed in months	0.89	0.77-1.04	0.14
Breastfed (exclusive/partial) in months	0.99	0.89-1.09	0.76
Maternal age at baseline (in years)	1.06	0.99-1.14	0.11

Legend

Figure 1: Mean predictive probability of peanut sensitization in children with primary peanut sensitization (Ara h 1,2 or 3 ≥0.35kU/L) versus children with peanut specific IgE ≥0.35kU/L but with negative results for primary peanut (Ara h 1,2 or 3<0.35kU/L).

Contributions of each author

HB, IK, EH, MW, GL had substantial contributions to the conception and design of the study. HB, KM, MP and VR made substantial contributions to acquisition of peanut-dust data and analysis. HB and AD performed the statistical analysis of results. IK, EH, CS, AB, EM and MW had substantial contribution to the BAMSE study and obtaining data to perform this study. CS performed the FLG genotyping. All authors contributed to the drafting and revising the manuscript for intellectual content and have approved the version to be published.

Conflict of interests

H. A. Brough has received research support, lecture fees, and travel support from ThermoFisher Scientific. G. Lack and V. Turcanu have received research support from the National Peanut Board. I Kull, K Makinson, E Hallner, C Söderhäll, A Douiri, M Penagos, E Melén, A Bergström and M Wickman declare that they have no relevant conflicts of interest.

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Environmental peanut exposure increases the risk of peanut sensitization in high risk children

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Word count: 3538~~2802~~

Short Title: Environmental peanut exposure and peanut sensitization in BAMSE

Abstract

Background: High household peanut consumption is associated with the development of peanut allergy, especially when peanut allergic cases are compared against atopic controls; thus environmental peanut exposure (EPE) may be a risk factor for peanut sensitization and allergy. In this study we explored the relationship between EPE and school-age peanut sensitization in a population based cohort.

Methods: Maternal bed-dust was collected postnatally and EPE was quantified using a polyclonal peanut ELISA. Peanut sensitization was assessed by ~~peanut~~ specific IgE to peanut extract and sIgE to peanut protein component allergens ~~molecule sensitizations (PAM) (~~Ara h 1, 2 or 3 ≥0.35kU/L (primary peanut sensitization)~~)~~. Initial nested case control analysis was performed comparing peanut sensitized cases against high-risk controls (matched for parental atopy) (n=411) using a conditional regression analysis. This was followed by whole cohort analysis (n=1878) comparing EPE against peanut sIgE sensitization at ages 4 and 8 years using Generalized Estimating Equations and against primary peanut ~~protein allergen molecule~~ sensitization at age 8 years using a logistic regression model. Finally, a subgroup analysis was performed comparing the impact of EPE in peanut sensitized versus egg-sensitized peanut tolerant individuals using logistic regression analysis. Levels of EPE were compared between groups using the Mann-Whitney U test.

Results: ~~There was a significant association between~~In the nested case control analysis, a higher level of ~~-~~EPE around birth was associated with ~~the presence of and~~ peanut specific IgE sensitization at age 4 years (OR=1.41, 95% CI:1.05-1.90), and primary peanut ~~protein allergen molecule~~PAM sensitization at age 8 years (OR=2.11, 95% CI:1.38-3.22) compared against high-risk controls. ~~*FLG* loss-of-function mutations, egg sensitization at age 4 years, infantile eczema and allergic rhinitis were significantly associated with peanut sensitization; however, there was no significant interaction with EPE.~~

When the whole BAMSE cohort was assessed, EPE was no longer associated with peanut sensitization; however, on subgroup analysis EPE was associated with primary peanut ~~protein allergen molecule~~PAM ~~-~~sensitization when compared against egg-sensitized peanut-tolerant controls

with an adjusted odds ratio of 1.4456 per unit EPE (95% CI:1.0614-1.9412). There was no significant interaction between EPE and *FLG* loss-of-function mutations, egg sensitization at age 4 years, infantile eczema or parental atopy on peanut sensitization.

Conclusions: Higher levels of environmental exposure to peanut in the first few months of life appear to ~~EPE was associated with an~~ increase the~~d~~ probability~~risk~~ of developing school age peanut sensitization in atopic children ~~specific IgE and peanut PAM sensitization at 8 years of age when comparing peanut sensitized cases against atopic controls~~ (based on egg sensitization and parental atopy).

~~(egg sensitized children or children matched for parental atopy).~~

Abstract word count: 3433 ~~251~~

Key words: BAMSE, egg sensitization, environmental peanut exposure, *FLG* mutation, infantile eczema peanut sensitization

Funding

The research was funded/supported by Action Medical Research (S/P/4529) and by the National Institute for Health Research (NIHR) Clinical Research Facility at Guy's & St Thomas' NHS Foundation Trust and NIHR Biomedical Research Centre based at Guy's and St Thomas' NHS Foundation Trust and King's College London. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health. The BAMSE study was funded by the Swedish Research Council, the Stockholm County Council, the Swedish Research Council for Health, Working life, and Welfare the Swedish Asthma and Allergy Foundation, the Swedish Heart and Lung Foundation, and the Gillbergska Foundation.

Abbreviations

AIC: Akaike information criterion

Ara h: Arachis hypogaea

BAMSE: Children/'Barn' Allergy Milieu Stockholm Epidemiological project

CI: confidence intervals

EPE: environmental peanut exposure

FLG: gene encoding the protein filaggrin

GEE: Generalized Estimating Equations

ISAAC: International Study of Asthma and Allergies in Childhood

IQR: Interquartile range

~~PAM: protein allergen molecule~~

QIC**:** Quasi Likelihood Independence models criterion

Introduction

Peanut allergy has increased,[1, 2] is a leading cause of anaphylaxis in food allergy[3] and has a significant impact on quality of life for the child and their family.[4, 5] Most children react on first known oral exposure to peanut; thus sensitization must be occurring earlier.[6, 7] Understanding the way children become sensitized to peanut is therefore imperative in order to prevent this condition. Observational[8] and animal work[9, 10] suggests that epicutaneous peanut exposure and early onset severe eczema may play an important role in peanut sensitization.

Epicutaneous exposure may be the route that peanut levels in dust sensitize a child in early life, particularly if the skin barrier is broken through eczema or specific genetic mutations associated with skin barrier dysfunction (e.g. filaggrin (*FLG)* mutations or eczema). We have recently shown in the Manchester Asthma and Allergy Study (MAAS) and Consortium of Food Allergy Research (CoFAR) cohort that *FLG* loss-of-function mutations and eczema severity respectively can increase the impact of early EPE on peanut sensitization and allergy.[11, 12]

A dose response relationship has also been demonstrated between household peanut consumption (used as an indirect marker for environmental peanut exposure: EPE) and the risk of developing peanut allergy in young children.[13] ~~They~~ Fox et al. compared household peanut consumption in peanut allergic individuals against children who were at high risk of developing peanut allergy (due to the presence of egg allergy) but had not developed peanut allergy. Household peanut consumption was ten times higher in households with infants with peanut allergy versus children with egg allergy without peanut allergy but was only 3 times higher in non atopic controls, highlighting that household peanut consumption was more likely to be associated with peanut allergy in high risk children.[13] Household peanut consumption and peanut sensitization has also been associated in other studies.[14] Peanut antigen in the infant's bed-dust and play-area-dust is highly positively correlated with household peanut consumption and stimulates basophils from peanut allergic children, thus has the potential to also sensitize individuals.[15]

Peanut sensitization (specific IgE≥0.35kU/L) is present in up to 10% of children and protein allergen molecule ~~(PAM)~~ analysis of the seed storage proteins in peanut (Ara h 1, 2 and 3) has been shown to improve the specificity and sensitivity of diagnosing peanut allergy.[16, 17] In BAMSE, children with IgE reactivity to Ara h 1, 2 or 3 at age 8 years (but not Ara h 8) reported peanut allergic symptoms in 87% of cases, whereas children with IgE reactivity exclusively to Ara h 8 reported peanut allergic symptoms in only 17% of cases which were also milder.[18] Sweden has high levels of birch pollen sensitization which explains the Bet v 1 in-vitro cross-reactivity with peanut Ara h 8;[18-20] thus, in this study we differentiated between primary peanut sensitization (IgE reactivity to Ara h 1, 2 or 3) and pollen cross-reactivity (IgE reactivity to Ara h 8).

This study aimed to assess whether EPE (as defined by peanut protein levels in household dust) is a risk factor for the development of school-age peanut sensitization. The analysis was done in two steps for financial and logistical reasons; firstly among high risk children (with parental atopy matched to peanut sensitised cases), in a nested cases analysis and secondly among the whole studied population (a cohort analysis). Given previous findings we also wanted to ascertain the modifying effects of *FLG* loss-of-function mutations, infantile eczema and preceding egg sensitization.

Methods

The BAMSE study is an unselected Swedish population birth cohort. The design and methodology are described elsewhere.[21] In brief 4089 unselected newborn children were recruited from 1994-1996 and have been evaluated for various health conditions over time. No intervention was performed. The BAMSE study obtained ethical approval for environmental sampling of dust.

Demographics

Serum specific IgE to peanut (ImmunoCAP system, ThermoFisher, Sweden) was measured at 4 and 8 years and children were defined as 'peanut sIgE sensitized' if peanut specific IgE was ≥0.35kU/L. Peanut protein component allergens ~~molecules~~ Ara h 1,2,3,8 and 9 ~~(PAM)~~ were assessed at 8 years (ImmunoCAP system, ThermoFisher, Sweden) in all children sensitized to peanut at age 8 years, irrespective of sensitization to birch. In addition, those children who were peanut sensitized at age 4 years but lost their sensitization at age 8 were also analyzed for these allergens. All other children with negative peanut sIgE at age 8 years were assumed to have negative component allergens to peanut. C~~and c~~hildren were considered to have primary ~~be~~ peanut ~~PAM protein allergen molecule~~ sensitization~~ed~~ if Ara h 1, 2 or 3 was ≥0.35kU/L and no~~t~~ primary peanut ~~protein allergen molecule PAM~~ sensitization~~ed~~ if Ara h 1, 2 and 3 were <0.35kU/L or peanut sIgE was <0.35kU/L at 8 years (where no sIgE peanut protein component allergens were ~~molecules~~~~PAM~~ available*)*. Twenty-four (1.3%) ~~C~~children with positive peanut specific IgE at 8 years of age but with no available serum for peanut protein component allergen ~~molecule~~~~PAM~~ analysis were excluded from this analysis. Egg sensitization was defined as egg specific IgE ≥0.35kU/L at 4 years of age. Previous allergic reactions to a food including itching in the mouth, nose/eye problems, trouble breathing, vomiting or diarrhoea, eczema or nettle rash was used from parental questionnaires at age 8 years.

Parental atopy was defined as a doctor's diagnosis of asthma and prescription of asthma medication and/or a doctor's diagnosis of hay fever in combination with furred pets- and/or pollen allergy at the time of questionnaire. This definition of parental atopy was selected rather than the

same definition with inclusion of a doctor's diagnosis of eczema (excluding contact dermatitis); this was because the children of parents with atopy including eczema were less atopic (lower infantile eczema) and parental atopy including eczema was not associated with the outcome of interest (primary peanut sensitization at 8 years of age); this is probably because adult eczema is often non-atopic.

Genotyping was performed for *FLG* mutations common in Scandinavia by using TaqMan allelic discrimination assays for R501X and R2447X and matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry for 2282del4. Children with a *FLG* mutation in any of these positions were classified as having a *FLG* loss-of function mutation.

A reported history of infantile eczema was assessed by a validated eczema questionnaire with sensitivity of 92% and specificity of 100%.[22] Eczema ~~and~~ was defined as dry skin, itchy rashes for 2 weeks or more and age specific localization (face or arms/legs extension surfaces or arms/legs flexures or wrists/ankles flexures) and/or doctor's diagnosis of eczema after 3 months and up to 1 year of age.~~during the first year of life.~~ Severity of eczema was assessed from questionnaires using three different measures:

1) Extent of localization of eczema (up to 10 areas) at or before their one year assessment

2) Topical steroids used as treatment for an itchy rash in the last year at their two year assessment (not available at their one year assessment)

3) Persistent eczema at age 8 years (using the same definition as above).

Ethnicity was based on where the parents were born. Allergic rhinitis at 4 and 8 years was assessed using International Study of Asthma and Allergies in Childhood (ISAAC) validated questionnaires, and was defined as persistent rhinitis without a common cold the last 12 months before 4 and 8 years respectively.[23]

Dust collection methodology

Dust samples were collected at a median of 2 months of age from the mother's mattress. The mother's mattress was vacuumed for 2.5 minutes with a small disposable filter bag (Allergy Control Products Inc. Ridgefield CT, USA) inserted in the front hose of the vacuum. The dust containing filter bags were sealed in plastic bags and stored at -20ºC. Dust samples were sieved and fine dust was weighed and extracted in proportional volumes of extraction solution as previously described.[24] Peanut protein in dust was determined using the Veratox polyclonal ELISA against whole peanut protein (Neogen Corporation, Lansing, MI, USA). The lower limit of quantitation (LLQ) of the assay was defined as 100ng/ml and samples below this value were defined as 50ng/ml (LLQ/2).[25] Results were converted from ng/ml into µg peanut protein/gram dust. Due to batch to batch variability of the Veratox ELISA results were batch corrected prior to being entered into the final statistical analysis.

Statistical analysis

Data was entered into an SPSS (SPSS 19.0; SPSS Inc, Chicago, IL, USA) and STATA spreadsheet (Timberlake Consultants Ltd London, UK) for the purpose of analysis. Peanut protein levels in dust (µg/gram) underwent natural log transformation. Initial nested case control analysis was performed with peanut sIgE sensitized cases at 4 years of age matched for gender and parental atopy with a 2:1 control (n=274) to case (n=137) matching. Children with primary peanut ~~protein allergen molecule~~PAM ~~-~~sensitization defined by Ara h 1, 2 or 3 ≥0.35kU/L at 8 years of age were ~~also~~ matched against controls at age 8 years with the same~~matched~~ sex~~for gender~~ and level of parental atopy with a 2:1 ratio of controls (n=130) to cases (n=65). Given that these controls were matched for the higher levels of parental atopy in the primary peanut sensitized cases these controls were deemed to be high-risk as the primary peanut sensitized cases had higher levels of parental atopy than the population overall (46.7% vs. 32.6%).Conditional logistic regression (incorporating matching) was performed for the case control analysis using robust standard error for peanut sIgE sensitization at 4 years and primary peanut sensitization at age 8 years.

Subsequently analysis was performed for the relationship between early EPE and peanut sensitization in all children from the BAMSE cohort with available postnatal maternal bed-dust and

FLG genotyping (n=1878). Logistic regression analysis was performed for primary peanut ~~protein allergen moleculePAM~~ sensitization at 8 years of age. Factors associated with peanut sIgE sensitization were assessed using Generalized Estimating Equations (GEE) with an exchangeable working correlation matrix to account for repeated measures within individuals at 4 and 8 years. Univariate followed by multivariate regression analysis was performed including EPE, ~~*FLG* loss-of-function mutations, infantile eczema and egg sensitization at 4 years of age~~ plus other covariates significantly associated with peanut sensitization ($P \leq .05$) which also improved the quality of fit of the multivariate model using the Akaike information criterion (AIC) for logistic regression and the Quasi Likelihood Independence models criterion (QIC) for GEE analyses.

Where the *FLG* mutation covariate was entered into the statistical model, participants with missing ethnicity data (35/1878=1.9%) and non-caucasians (165/1842=8.8%) were excluded as distinct *FLG* loss-of-function mutations are present in different populations and the *FLG* loss-of-function mutations assessed in this study have only been associated with eczema in Caucasian European populations [26, 27] Peanut levels in dust (µg/g) were compared between groups using the Mann-Whitney U test. ~~Proportions between groups (e.g. infantile eczema and *FLG* mutations) were compared using Pearson chi-squared.~~ Statistical significance was assessed at $P < .05$.

Results

Details of demographics and clinical characteristics included on this study are described in Table EI. Median peanut protein [IQR] in dust was 4.07µg/gram [1.58, 11.86]. Peanut extract sIgE sensitization was 6.6% at 4 years (n=103/1572) and 8.6% at 8 years (n=161/1876). Primary pPeanut protein allergen moleculePAM sensitization at 8 years was 4.1% (n=75/1854), of which 74/75 (99%) had Ara h 2 ≥0.35kU/l. We first performed a nested case control analysis, and based on positive findings for this subsequently proceeded to then analyse the whole BAMSE cohort.

Nested case control analysis for peanut sIgE and primary peanut protein allergen moleculePAM sensitization

We compared peanut sensitized children against children without peanut sensitization matched for gender and parental atopy as described in the statistical methods; EPE was a risk factor for both peanut extract sIgE sensitization at 4 years and primary peanut protein allergen moleculePAM sensitization at 8 years with a 23% and 29% increased probability of developing risk of peanut sensitization per natural log (*ln*) unit increase in EPE respectively (Table I). In children who were peanut extract sIgE sensitized but not peanut protein allergen moleculePAM sensitized to peanut protein component allergens Ara h 1, 2 and/or 3, there was only borderline significance towards an association between EPE and peanut sensitization (OR=1.20, 95% CI: 0.97-1.48).

Differential relationship between EPE and primary peanut protein allergen moleculePAM sensitization and non-clinically significant peanut sensitization is displayed in Figure 1. Median peanut protein in dust was higher in peanut extract sIgE sensitized children (3.39 µg/g, IQR 1.41-11.01, n=137) than non-peanut extract sIgE sensitized controls (2.28µg/g, IQR 0.88-5.14, n=274) (*P*<.01); and higher in primary peanut protein allergen moleculePAM sensitized children (4.64µg/g, IQR 1.58-12.77, n=65) than in control children that were not sensitized to Ara h 1,2 or 3 non-peanut protein allergen moleculePAM sensitized controls (2.24µg/g, IQR 0.88-4.74, n=130) (*P*<.01).

On multivariate conditional logistic regression analysis, EPE was significantly associated with peanut specific IgE and primary peanut protein allergen moleculePAM sensitization with a 1.41- and

2.1-fold increased probability of developing peanut sensitization ~~risk~~ per unit increase EPE respectively (Table II).

Infantile eczema and egg sensitization were both significantly associated with sensitization to peanut extract ~~sIgE~~ and ~~protein allergen moleculePAM~~ sensitization to peanut protein component allergens Ara h 1, 2 and/or 3.(Table II). *FLG* loss-of function mutations increased the ~~risk of~~probability of developing peanut specific IgE sensitization 3.78-fold and primary peanut ~~protein allergen moleculePAM~~ sensitization 7.33-fold. Allergic rhinitis at age 4 and 8 years was associated with both the probability of developing peanut specific IgE sensitization at age 4 years and primary peanut ~~protein allergen moleculePAM~~ sensitization at age 8 years in the multivariate analysis. The number of biological siblings, duration of exclusive and total breastfeeding and maternal age at baseline were not ~~risk factors~~associated with ~~for~~ peanut sensitization. There was no significant interaction between EPE and either infantile eczema, egg sensitization or *FLG* loss-of-function mutations on primary peanut ~~protein allergen moleculePAM~~ sensitization (data not shown).

Whole cohort analysis for peanut extract sIgE sensitization and primary peanut sensitization~~protein allergen moleculePAM sensitization~~

In a second step following positive findings obtained on case control analysis, we assessed~~analysis was performed for~~ the relationship between early EPE and peanut sensitization in all children from the BAMSE cohort with available postnatal maternal bed-dust and *FLG* genotyping (n=1878). On both univariate (Table III) and multivariate analysis (Table IV) infantile eczema, egg sensitization at age 4 years, tree nut and soya allergy at age 8 years, *FLG* loss-of function mutation and allergic rhinitis at age 8 years were associated with the probability of peanut ~~extract~~ sIgE sensitization at age 4 and 8 years. For primary peanut ~~protein allergen moleculePAM~~ sensitization at age 8 significant associations were also seen on univariate (Table III) and multivariate analysis (Table IV) for infantile eczema, egg sensitization, tree nut and soya allergy at age 8 years and allergic rhinitis at age 8 years. Although measures of eczema severity several other food allergies at age 8 years

were significantly association with peanut sensitization on univariate analysis, these variables were no longer significantly associated on multivariate analysis. ~~However,~~ EPE was not ~~longer~~ associated with peanut sIgE sensitization or primary peanut ~~protein allergen molecule~~ sensitization. There was no interaction between EPE and *FLG* mutations, infantile eczema, egg sensitization, allergic reaction to tree nut or soya by age 8 years, or parental atopy on peanut sensitization.

Subgroup analysis of primary peanut ~~protein allergen molecule~~~~PAM~~ sensitization against egg sensitized non peanut allergic individuals

On subgroup analysis we compared primary peanut ~~protein allergen molecule~~PAM sensitized children (n=75) against children who were sensitized to egg (sIgE≥0.35kU/L at age 4 years) but did not go on to ~~develop~~ have primary peanut ~~protein allergen molecule~~PAM sensitization at age 8 years (n=59). EPE increased the probability ~~was a risk factor for~~of primary peanut ~~protein allergen molecule~~PAM sensitization on both univariate (OR=1.33 95% CI:1.01-1.74, *P*<.05, n=134) (Table V) and multivariate analysis (OR=1.44~~56~~, 95% CI:1.06~~14~~-1.94~~2.12~~, P0~~<~~.018, n=133~~2~~, AIC: 167~~331~~) adjusting for ~~allergic rhinitis at age 8 years and~~ infantile eczema and allergic reaction to soya by age 8 years. Peanut levels in household dust were also significantly higher in primary peanut ~~protein allergen molecule~~PAM sensitized children (median 4.79µg/g, IQR 1.63-12.00) versus egg sensitized, non-primary peanut ~~protein allergen molecule~~PAM sensitized children (median 2.35µg/g, IQR 1.23-6.15) (*P*=.03).

We assessed the impact of EPE on peanut sensitization when using other high-risk groups of children who were not ~~did not become~~ peanut sensitized at age 8 years (*FLG* mutations, infantile eczema, soya allergy and ~~,~~ parental atopy) and there was no significant association.

Discussion

In the prospective birth cohort BAMSE, there was an exposure-response relationship between EPE (quantified using peanut protein levels from maternal mattress-dust postnatally) and primary peanut ~~protein allergen molecule~~~~PAM~~ sensitization (Ara h 1, 2 or 3 ≥0.35kU/L) among high risk children (in a nested cases analysis). When all children in the whole studied population were assessed, there was no association between EPE and peanut sensitization; however, on subgroup analysis there was an exposure-response relationship between EPE and primary peanut ~~protein allergen molecule~~~~PAM~~ sensitization when compared against high risk egg sensitized children.

~~This supports the findings by~~ Fox et al. also showed that ~~where~~ household peanut consumption during the first year of life (as an indirect marker of EPE) was higher in peanut allergic cases than in egg allergic controls.[13] In the nested cases analysis ~~t~~The exposure-response relationship ~~between EPE and PS (when compared against atopic controls)~~ was stronger between EPE and primary peanut ~~protein allergen molecule~~~~CRD~~ sensitization (Ara h 1,2 or 3≥0.35kU/L) than between EPE and non-clinically relevant peanut sensitization (peanut sIgE ≥0.35kU/L but negative results for Ara h 1,2 or 3<0.35kU/L) (Figure 1). This supports the concept that EPE increases the probability of developing clinically relevant peanut sensitization which is more likely to develop into peanut allergy rather than non-specific peanut sensitization.

~~The association between household peanut consumption and peanut sensitization was also found in a study by Garcia-Boyano et al.[14] and supports the concept that EPE increases the risk of clinically relevant peanut sensitization.~~

In other cohorts we have shown a synergistic effect between EPE and markers of an impaired skin barrier (infantile eczema and *FLG* loss-of-function mutations) on peanut sensitization and allergy.[11, 12] Although in BAMSE we did not find significant interactions between EPE and *FLG* mutations or infantile eczema on peanut sensitization, we did show on subgroup analysis that early EPE has more impact on the probability of developing peanut sensitization when compared against high risk

children (defined by parental atopy in the nested cases analysis and egg sensitization in the whole cohort analysis) ~~in children with certain risk factors for the development of peanut sensitization (such as parental atopy or egg sensitization)~~ than in children without these risk factors. The BAMSE cohort is relatively non-atopic, being from the general population in Sweden, which may have contributed to lower strength of associations between EPE and atopic markers. The median level of peanut protein in maternal bed-dust dust in the BAMSE cohort was 4.07µg/gram [IQR 1.58, 11.86], similar to that found in another the UK study by our group (median 4.19 IQR 0.54-24.89). [24] The level of peanut in dust was higher in a US study that measured peanut from the living room floor (median 39.1ug/g IQR 0.4-1.33). [(10)] It would; however, be expected that peanut levels would be higher in America due to higher levels of peanut consumption.

Infantile eczema was one of the most important risk factors for peanut sensitization in BAMSE; this association has been cited in numerous studies.[8, 28] ~~*FLG* loss of function mutations were less important than infantile eczema in predicting the development of peanut sensitization, and *FLG* mutations became more important if infantile eczema was not included in the regression model. Previous studies have found that children that carry a *FLG* mutation have an increased risk of peanut allergy even after adjusting for eczema.[29] In this study eczema seemed to be the overriding factor for peanut sensitization; however, it should be noted that *FLG* mutations were quite low in BAMSE (7.14%) in comparison with other population based cohorts.[26, 29]~~ Measures of eczema severity (extent of localisation, topical steroid use and persistent eczema) were associated with peanut sensitization on univariate analysis but were no longer associated on multivariate analysis. Eczema severity has previous been shown to be an independent risk factor for food sensitization in the EAT (Enquiring About Tolerance) study.[30] The EAT study used the SCORAD eczema severity scoring system; however, this could not be performed in the BAMSE study as the evaluations at 1, 2, 4 and 8 years were based on parental questionnaires.

Egg sensitization was highly associated with peanut sensitization; which has previously been shown in numerous cohort studies;[11, 12, 31] it may be that egg sensitization shows a predisposition to mount allergic antibodies to other allergens as egg allergy is highly associated with the development of other food allergies, asthma and aeroallergen sensitization.[32, 33] Observational studies have shown that in children with severe, early onset eczema, up to 50% are sensitized to egg;[28] thus egg sensitization may be an additional marker of eczema severity. *FLG* loss-of-function mutations were less important than infantile eczema in predicting the development of peanut sensitization, and *FLG* mutations became more important if infantile eczema was not included in the regression model. Previous studies have found that children that carry a *FLG* mutation have an increased risk of peanut allergy even after adjusting for eczema.[29] In this study eczema seemed to be the overriding factor for peanut sensitization; however, it should be noted that *FLG* mutations were quite low in BAMSE (7.14%) in comparison with other population-based cohorts.[26, 29, 34] Tree nut and/or soya allergy by age 8 was also associated with primary peanut sensitization at age 8 years; co-existent peanut and tree-nut allergy has been shown in numerous studies,[35, 36] whereas co-existent soya and peanut allergy is reportedly low.[37]

Strength of the present study includes the population based design and relatively high number of study participants. Limitations of this study included the lack of diagnostic food challenges to determine peanut allergy as the BAMSE study was not originally designed to evaluate peanut allergic outcomes; however, primary peanut ~~protein allergen molecule~~PAM sensitization has been shown to be a much more accurate predictor of clinically confirmed peanut allergy than peanut specific IgE.[38, 39] Certain potential confounders were not available such as parental peanut allergy or other parental food allergies, maternal peanut consumption during pregnancy and breast-feeding and infant peanut consumption. Therefore we are unable to assess whether infant peanut consumption could have protected the children against environmental exposure to peanut ~~sensitization~~ as has been shown in previous studies;[13, 35] however, when BAMSE was recruiting participants in 1996 the number of infants eating peanut was very low. Caucasian ethnicity was defined based on place of parental birth rather than self-reported ethnicity; however, when the

BAMSE study was recruiting participants 1994-1996, most parents born in Sweden or Northern Europe would have been of Caucasian descent. ~~as being born in Sweden as between 1994-1996 (when the BAMSE cohort commenced), most participants born in Sweden would have been of Northern European descent. In addition participants from Finland, Greece or Eastern Europe who were not born in Sweden were also defined as Caucasian~~.[40] Findings on case control analysis could not be replicated on whole cohort analysis, therefore these findings need to be confirmed in other population based cohorts. ~~Strength of the present study includes the population based design and relatively high number of study participants.~~

Conclusion

In summary, although EPE did not increase the risk of peanut sensitization in children who did not already have atopic risk factors, the findings from the BAMSE cohort support the hypothesis that in specific high risk groups, peanut levels in dust around the time of birth may pose a risk for later peanut sensitization. Further prospective studies are needed to confirm these findings in other populations.

Acknowledgement

We wish to thanks Dr Alick Stephens for his assistance in the BAMSE dust analysis. The authors thank all children and their parents in the BAMSE cohort

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Table EI: Demographics of whole studied BAMSE population with available dust and *FLG* genotyping (n=1878)

	Number of cases	No. data points	Percentage of cases / no. of available data points
Peanut specific IgE ≥ 0.35kU/L at 4 years	103	1572	6.55%
Peanut specific IgE ≥ 0.35kU/L at 8 years	161	1876	8.58%
Ara h 1, 2 or 3 ≥0.35kU/L at 8 years vs. Ara h 1, 2 or 3 <0.35kU/L or peanut sIgE <0.35kU/L (excluding peanut sIgE ≥0.35kU/L with no components or missing data) at 8 yrs	75	1854	4.05%
History of infantile atopic dermatitis	313	1840	17.01%
Topical steroid use between age 1-2 years	534	1842	29.0%
Higher extent of localisation of eczema (>5 areas vs. <5 areas or no eczema)	49	1841	2.7%
Persistent eczema at age 8 years	321	1828	17.6%
At least one *FLG* mutation (R501X, 2282del or R2447x)	134	1878	7.14%
Allergic rhinitis (ISAAC definition) (age 4 years)	215	1828	11.76%
Allergic rhinitis (ISAAC definition) (age 8 years)	288	1865	15.44%
Egg sIgE≥ 0.35IU/ml at 4 years of age	82	1575	5.21%
Reported allergic reaction to any food 8 years	349	1869	18.7%
Reported allergic reaction to peanut by 8 years	120	1869	6.4%
Reported allergic reaction to milk by 8 years	53	1869	2.8%
Reported allergic reaction to egg by 8 years	36	1869	1.9%
Reported allergic reaction to fish by 8 years	12	1869	0.6%
Reported allergic reaction to shellfish 8 years	17	1869	0.9%
Reported allergic reaction to wheat by 8 years	9	1869	0.5%
Reported allergic reaction to soya by 8 years	21	1869	1.1%
Reported allergic reaction to apple by 8 years	83	1869	4.4%
Reported allergic reaction to peach by 8 years	44	1869	2.4%
Reported allergic reaction to kiwi by 8 years	92	1869	4.9%
Reported allergic reaction to avocado 8 years	14	1869	0.7%

Reported allergic reaction to banana by 8 years	17	1869	0.9%
Reported allergic reaction to carrot by 8 years	53	1869	2.8%
Reported allergic reaction to tree nuts 8 years	105	1869	5.6%
Male gender	973	1871	52.00%
Caucasian ethnicity	1677	1842	91.04%
Biological siblings	1695	1870	90.64%
Parental atopy – (asthma/AR/eczema~~AD~~)	838	1858	45.10%
Parental atopy – (asthma/AR)	605	1856	32.60%
Maternal age (mean, SD)	30.9 (SD 4.49)	1871	N/A
Peanut protein in maternal bed-dust (µg/g): median [IQR]	4.07 [1.58-11.86]	1878	N/A
Exclusive breast-feeding duration (months): mean (SD)	5.19 (2.43)	1838	N/A
Total breast-feeding duration (months): mean (SD)	8.73 (3.31)	1825	N/A

Table I: Univariate conditional logistic regression incorporating matching for peanut sensitized cases versus non-sensitized controls matched for gender and parental atopy. Peanut sIgE sensitization (≥0.35kU/L) at age 4 years and primary peanut ~~protein allergen moleculePAM~~ sensitization (Ara h 1, 2 or 3 ≥0.35kU/L) at age 8 years.

	Peanut sIgE sensitized at age 4 years (n=411)			Peanut PAM sensitized at age 8 years (n=195)		
	OR	95% CI	*P* value	OR	95% CI	*P*-value
EPE (µg/gram ln transformed)	1.23	1.06-1.43	**<.01**	1.29	1.04-1.61	**.02**
No. of biological siblings	1.38	0.63-3.03	.42	2.50	0.77-8.16	.13
≥1 *FLG* loss-of-function mutation (excluding non-caucasians)	2.67	1.18-6.04	**.02**	3.00	0.84-10.75	.09
History of infantile eczema	7.35	4.20-12.83	**<.001**	6.39	2.95-13.83	**<.001**
Egg sIgE sensitization: 4 years	18.91	7.50-47.66	**<.001**	12.85	3.78-43.67	**<.001**
*Allergic rhinitis: 4 years (ISAAC)	3.44	1.90-6.23	**<.001**	2.53	1.04-6.18	**.04**
*Allergic rhinitis: 8 years (ISAAC)	11.44	5.68-23.07	**<.001**	12.97	4.54-37.05	**<.001**
Exclusively breastfed (months)	0.95	0.88-1.03	.22	0.98	0.87-1.11	.77
Breastfed (excl/partial) (months)	0.99	0.93-1.05	.74	1.01	0.93-1.11	.76
Maternal age at baseline (in years)	0.99	0.94-1.04	.65	1.05	0.99-1.12	.12

*Allergic rhinitis was included to assess whether false positive results for peanut sensitization were being found due to cross-reactivity with grass or tree pollens.

Table II: Multivariate conditional logistic regression incorporating matching for peanut sensitized cases versus non-sensitized controls matched for gender and parental atopy. Peanut sIgE sensitization (≥0.35kU/L) at age 4 years and primary peanut ~~protein allergen moleculePAM~~ sensitization (Ara h 1, 2 or 3 ≥0.35kU/L) at age 8 years. Non-caucasians were excluded due to inclusion of *FLG* mutations as a covariate.

	Peanut sIgE sensitized at age 4 years (excluding non-caucasians) (n=237) *AIC: 90.78			Primary p~~P~~eanut ~~PAM~~ sensitization~~ed~~ at age 8 years (excluding non-caucasians) (n=143) *AIC: 49.19		
	OR	95% CI	*P* Value	OR	95% CI	*P* Value
EPE (µg/gram ln transformed)	1.41	1.05-1.90	**.02**	2.11	1.38-3.22	**.001**
≥1 *FLG* loss-of-function mutation	3.78	0.93-15.36	.06	7.33	1.21-44.21	**.03**
History of infantile eczema	2.53	0.98-6.51	**.05**	3.93	1.03-15.00	**.05**
Egg sIgE sensitization: 4 years	28.00	5.24-149.39	**<.001**	39.9	1.47-1081.68	**.03**
**Allergic rhinitis: 8 years (ISAAC)	12.57	4.164-37.98	**<.001**	28.93	3.77-221.61	**<.001**

*Smaller AIC is better. **Allergic rhinitis at age 8 years was selected as it improved the fit of the model (AIC better than allergic rhinitis at age 4 years).

Table III: Univariate analysis of peanut specific IgE sensitization aged 4 and 8 years using GEE to account for repeated measures within individuals at 4 and 8 years and logistic regression for primary peanut ~~PAM~~ sensitization at age 8 years in the whole BAMSE cohort (n=1878).

	GEE for peanut sIgE Univariate peanut sIgE sensitisation at 4 +8 years			Univariate primary peanut sensitisation at age 8 years		
	OR	95% CI	*P* Value	OR	95% CI	*P* Value
EPE (µg/gram ln transformed)	0.95	0.86-1.06	0.39	1.01	0.86-1.19	.89
Gender	1.14	0.83-1.56	0.43	1.41	0.88-2.26	.15
Full older siblings	1.08	0.63-1.85	0.77	0.86	0.40-1.81	0.68
≥1 *FLG* loss-of-function mutations	1.84	1.10-3.05	**0.02~~19~~**	1.84	0.89-3.77	**0.10**
Questionaire based history of infantile eczema	6.49	4.66-9.02	**<.001**	7.94	4.91-12.83	**<.001**
Localisation of infantile eczema (continuous 1-10 areas/per unit)	1.48	1.37-1.59	**<.001**	1.53	1.40-1.68	**<.001**
Localisation of infantile eczema (<5 areas vs. 5+ areas)	7.69	4.26-13.55	**<.001**	10.4	5.24-20.65	**<.001**
Application of topical steroids for itchy rash (1-2 yrs)	3.24	2.35-4.46	**<.001**	3.95	2.46-6.34	**<.001**
Persistent eczema at age 8 years	3.87	2.76-5.44	**<.001**	2.85	1.69-4.81	**<.001**
Egg sIgE sensitisation at 4 years	13.58	8.55-21.58	**<.001**	11.04	6.13-19.90	**<.001**
Allergic reaction to milk by 8 years	5.68	3.12-10.34	**<.001**	4.24	1.84-9.77	**0.001**
Allergic reaction to egg by 8 years	26.81	13.98-51.4	**<.001**	16.67	7.81-35.59	**<.001**
Allergic reaction to fish by 8 years	17.25	5.41-54.96	**<.001**	9.18	2.39-35.32	**0.001**
Allergic reaction to shellfish 8 years	15.59	6.07-40.02	**<.001**	3.70	0.82-16.71	0.09
Allergic reaction to wheat by 8 years	9.55	2.53-36.02	**0.001**	8.06	1.60-40.59	**0.01**
Allergic reaction to soya by 8 years	48.75	17.61-134.97	**<.001**	41.95	16.56-106.24	**<.001**

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Allergic reaction to apple by 8 years	12.54	8.16-19.27	**<.001**	5.93	3.15-11.13	**<.001**
Allergic reaction to peach by 8 years	14.37	8.15-25.34	**<.001**	6.49	2.88-14.61	**<.001**
Allergic reaction to kiwi by 8 years	5.95	3.80-9.31	**<.001**	4.25	2.20-8.20	**<.001**
Allergic reaction to avocado 8 years	12.27	4.61-32.66	**<.001**	4.38	0.95-20.13	**0.06**
Allergic reaction to banana 8 years	12.19	5.07-29.30	**<.001**	3.44	0.77-15.40	0.11
Allergic reaction to carrot by 8 years	17.84	10.6-30.05	**<.001**	7.83	3.83-16.03	**<.001**
Allergic reaction to tree nuts 8 years	21.93	14.45-33.28	**<.001**	10.29	12.08-34.05	**<.001**
Allergic rhinitis aged 4 years	2.82	1.92-4.13	**<.001**	2.16	1.22-3.83	**0.008**
Allergic rhinitis aged 8 years	8.05	5.78-11.22	**<.001**	5.87	3.66-9.42	**<.001**
Parental atopy (asthma and/or AR)	1.77	1.28-2.44	**<.001**	1.77	1.10-2.83	**0.018**
Parental atopy (asthma, AR,eczema)	1.44	1.05-1.98	**0.02**	1.50	0.95-2.39	0.09
Non-caucasian ethnicity	1.58	0.97-2.57	0.07	1.09	0.49-2.43	0.79
Exclusively breastfed in months	0.98	0.94-1.06	0.92	0.97	0.88-1.06	0.49
Breastfed (exclusive/partial) in mo	0.99	0.94-1.03	0.57	0.97	0.91-1.04	0.43
Maternal age at baseline (in years)	1.02	0.98-1.05	0.44	1.02	0.97-1.07	0.53

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Table IV: Multivariate analysis of peanut specific IgE sensitization aged 4 and 8 years using generalised estimating equations (GEE) to account for repeated measures within individuals at 4 and 8 years and logistic regression analysis for primary peanut ~~PAM~~ sensitization at age 8 years in the BAMSE cohort. ~~excluding non-caucasians.~~ Goodness of fit assessed by QIC and AIC.

	Multivariate peanut sIgE sensitization (excluding non-caucasians) (136788) QIC:935.41094			Multivariate primary peanut ~~PAM~~ sensitization ~~(excluding non-caucasians)~~ (n=1521375) AIC:386767		
	OR	95% CI	*P* Value	OR	95% CI	*P* Value
EPE (µg/gram ln transformed)	0.948	0.8084-1.113	.4575	1.088	0.878-1.342	.48
≥1 *FLG* loss-of-function mutations	2.5237	1.242-5.104.60	**0.01**	~~1.82~~	~~0.72-4.57~~ ns	~~.20~~
History of infantile eczema	2.753.81	1.672.44-4.485.94	**<.001**	4.025.51	2.14-7.543.03-10.03	**<.001**
Tree nut allergic reaction by age 8 years	9.30	4.94-17.53	**<.001**	4.80	2.38-9.66	**<.001**
Soya allergic reaction by age 8 years	18.21	4.75-69.80	**<.001**	2.54	3.85-42.07	**<.001**
Egg sIgE sensitization at 4 years	7.668.12	3.924.35-14.955.15	**<.001**	4.246.61	1.98-9.093.16-13.80	**<.001**
Allergic rhinitis aged 8 years (ISAAC)	4.025.57	2.493.57-6.528.71	**<.001**	2.013.05	1.0565-3.835.63	**0<.0341**

ns: Not significant on multivariate analysis therefore not included in final multivariate analysis.

Table V: Univariate analysis of factors associated with primary peanut ~~PAM~~ sensitization (at age 8 years) versus non primary peanut ~~PAM~~ sensitization (at age 8 years) in high risk children with preceding egg sensitization (at age 4 years) (n=134).

	Primary peanut ~~PAM~~ sensitization vs. non-peanut sensitized egg sensitized controls		
	OR	95% CI	*P* Value
EPE (µg/gram ln transformed)	1.33	1.01-1.74	**<0.05**
Gender	1.90	0.95-3.80	0.07
Full older siblings	0.61	0.17-2.13	0.44
≥1 *FLG* loss-of-function mutations (excluding non-caucasians)	1.09	0.33-3.56	0.89
History of infantile eczema	2.51	1.24-5.08	**0.01**
Localisation of infantile eczema (continuous 1-10 areas/per unit)	1.15	1.01-1.32	**0.04**
Localisation of infantile eczema (<5 areas vs. 5+ areas)	1.36	0.52-3.53	0.53
Application of topical steroids for itchy rash (1-2 yrs)	1.40	0.70-2.80	0.34
Persistent eczema at age 8 years	2.32	1.09-4.90	**0.03**
Allergic reaction to milk by 8 years	1.42	0.39-5.09	0.59
Allergic reaction to fish by 8 years	0.78	0.15-4.00	0.78
Allergic reaction to shellfish 8 years	0.30	0.06-1.58	0.16
Allergic reaction to wheat by 8 years	1.59	0.14-17.96	0.71
Allergic reaction to soya by 8 years	11.05	1.39-87.63	**0.02**
Allergic reaction to apple by 8 years	1.71	0.64-4.52	0.29
Allergic reaction to peach by 8 years	1.29	0.40-4.17	0.67
Allergic reaction to kiwi by 8 years	1.42	0.52-3.85	0.50

Allergic reaction to avocado 8 years	0.78	0.11-5.71	0.81
Allergic reaction to banana 8 years	0.38	0.07-2.13	0.27
Allergic reaction to carrot by 8 years	2.36	0.71-7.85	0.16
Allergic reaction to tree nuts 8 years	3.43	1.54-7.62	**0.002**
Allergic rhinitis aged 4 years (ISAAC)	0.87	0.38-1.96	0.73
Allergic rhinitis aged 8 years (ISAAC)	2.65	1.26-5.56	**0.01**
Parental atopy (asthma/ and /or hay-fever)	1.34	0.67-2.65	0.41
Non-caucasian ethnicity	2.55	0.93-6.98	0.07
Exclusively breastfed in months	0.89	0.77-1.04	0.14
Breastfed (exclusive/partial) in months	0.99	0.89-1.09	0.76
Maternal age at baseline (in years)	1.06	0.99-1.14	0.11

Legend

Figure 1: Mean predictive probability of peanut sensitization in children with primary peanut ~~PAM~~ sensitization (Ara h 1,2 or 3 ≥0.35kU/L) versus children with peanut specific IgE ≥0.35kU/L but with negative results for primary peanut ~~PAM~~ (Ara h 1,2 or 3<0.35kU/L).

Contributions of each author

HB, IK, EH, MW, GL had substantial contributions to the conception and design of the study. HB, KM, MP and VR made substantial contributions to acquisition of peanut-dust data and analysis. HB and AD performed the statistical analysis of results. IK, EH, CS, AB, EM and MW had substantial contribution to the BAMSE study and obtaining data to perform this study. CS performed the FLG genotyping. All authors contributed to the drafting and revising the manuscript for intellectual content and have approved the version to be published.

Conflict of interests

H. A. Brough has received ~~research support from the National Peanut Board and has received~~ research support, lecture fees, and travel support from Thermo-Fisher Scientific. G. Lack and V. Turcanu have received research support from the National Peanut Board. I Kull, K Makinson, E Hallner, C Söderhäll, A Douiri, M Penagos, E Melén, A Bergström and M Wickman declare that they have no relevant conflicts of interest.

Comment [HB1]: Research funds went through Gideon Lack and Turcanu not Helen Brough

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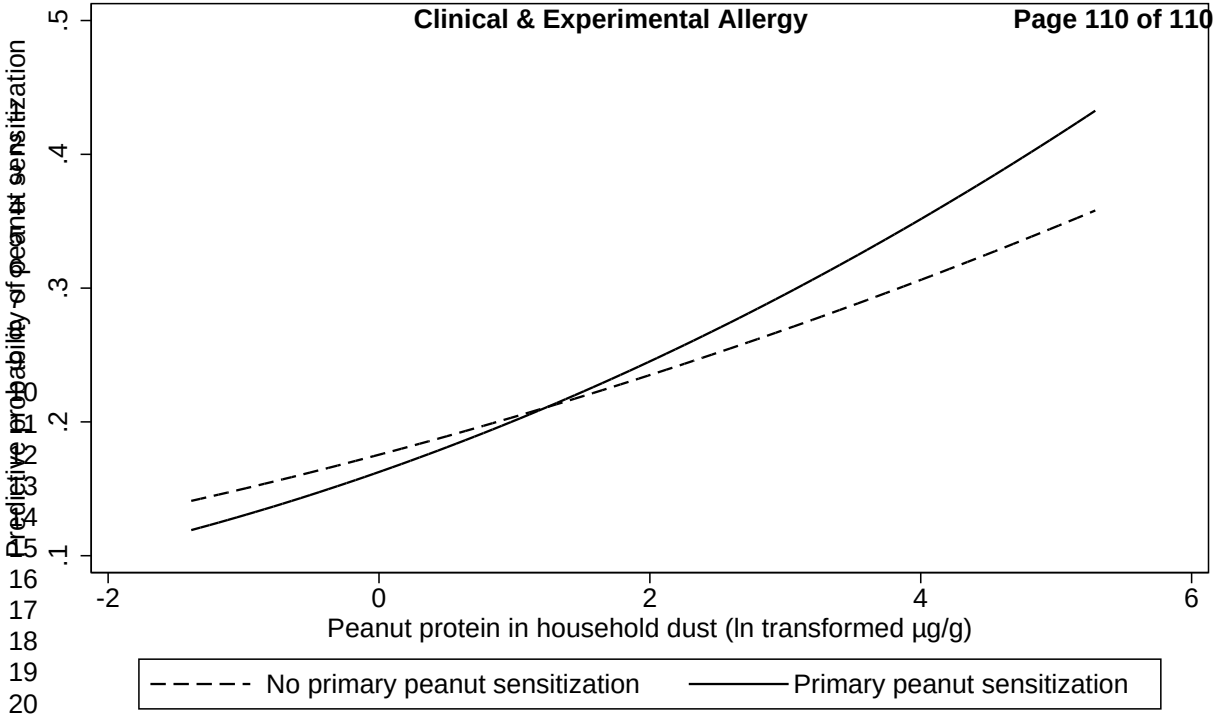
Predictive probability of peanut sensitization
.1
.2
.3
.4
.5
-2
0
2
4
6
Peanut protein in household dust (ln transformed µg/g)
No primary peanut sensitization
Primary peanut sensitization